GROWTH AND TISSUE CULTURE STUDIES ON REGENERATION POTENTIAL OF SOME ECONOMIC MEDICINAL CROP PLANTS

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BY

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MOST HUMBLY DEDICATED

TO

MY RESPECTED PARENTS

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Certificate

*This is to certify that the work present in this dissertation entitled "**Growth and tissue culture studies on regeneration potential of some economic medicinal crop plants**" is the original piece of work carried out by **MS. Nahida Tun Nisa Chishti** under my guidance and supervision and has not been submitted elsewhere for the award of any other degree or diploma and can be submitted in partial fulfilment of the requirements for the award of the degree of **Master of Philosophy in Botany**.*

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ABBREVIATIONS

BAP/BA	- 6-Benzyl amino purine
$CaCl_2.2H_2O$	- Calcium Chloride
cm	- centimeter.
$CoCl_2.6H_2O$	- Cobalt Chloride
Conc.	- Concentration
$CuSO_4.5H_2O$	- Copper sulphate
cv	- Cultivar
cw	- Coconut Water
2,4-D	- 2,4-dichlorophenoxy acetic acid.
DDW	- Double distilled water
EDTA	- Ethylene diamine tetra acetic acid
Fig.	- Figure
gm	-gram
gml^{-1}	-gram per litre
Hist.	-Histogram
IAA	-Indole 3-acetic acid
IBA	-Indole 3-butyric acid
KH_2PO_4	-Potassium dihydrogen Orthophosphate
KI	-Potassium iodide
Kn	-Kinetin
KNO_3	-Potassium nitrate
l	-litre
LS	-Linsmaier and Skoog's medium
mg	-milligram
mgl^{-1}	-milligram per litre
µM	-Micromole

MS	-Murashige and Skoog's medium
$MnSO_4.4H_2O$	-Manganese sulphate
$Na_2MoO_4.2H_2O$	-Sodium molybdate
NAA	-Napthalene acetic acid
NaCl	-Sodium Chloride
NH_4NO_3	-Ammonium nitrate
uv	-Ultra Violet
1x	-Normal Solution
10x	10 times stronger solution
$ZnSO_4.7H_2O$	-Zinc Sulphate

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INTRODUCTION

CHAPTER-1

INTRODUCTION

Tissue culture is a loose term used for the *in vitro* culture of various explants. Recent progress in plant cell and tissue culture has turned this area of research into one of the most dynamic and promising fields of experimental biology .The importance and applications of plant tissue culture in plant science are quite vast and varied. There have been many valuable contributions of plant tissue culture in the fields of fundamental, academic and applied Botany.(Murashige, 1980). The techniques of tissue culture have attracted the attention of scientist from all disciplines of science as they form a very useful tool for solving many present day problems. We are now able to culture single cell to raise whole plant from isolated meristem or to induce callus to develop into complete plant either by organogenesis or directly by embryogenesis *in vitro*.

More immediately, however the availability of medicinal plants is under serious threat. About 95% of the medicinal plants used by Indian industries today are collected as their wild forms. Less than 20 plants of species are under commercial cultivation. Over 70% of the plant collections involve destructive harvesting because of the use of whole plants (in case of herbs). This creates a definite threat to the genetic stocks and to the medicinal plants in particular. Conservation of medicinal plants species is possible through either *in situ* and or *ex situ* means.

The recent development in clonal propagation of plants through tissue culture techniques has been of great help in the cultivation of medicinal plants by providing planting material of standard quality and it was conceived and enunciated by Haberlandt (1902). Recent advances in biotechnology for producing transgenic plants have added a new dimension to plants as a source of pharmaceutical products and also it is possible to

enhance the products within a short period of time by manipulating precursor and growth regulators.

1.1 ROLE OF PLANTS IN HUMAN LIFE

The importance of plants to the living world hardly requires any introduction, Man started tailoring them ever since the beginning of civilization in many ways suitable to his requirements Mans basic requirements are three i.e. food, shelter and clothing. All these requirements have been accomplished by plants from prehistoric time. Batra (1995) pointed out the obvious role of plants in human life. Modern civilization despite its far reaching advancement still depends basically and largely on plant products. Thus, the scope of plant science and its important consequences are vast and immense.

1.2 IMPORTANCE OF TISSUE CULTURE

Currently, a new technology, called biotechnology is receiving the topmost priority all over the world and aiming at revolutionizing different walks of life mainly medicine, industry, agriculture, environment etc. Now, tissue culture has become an integral part of biotechnology. Therefore, let us start with the term "Plant Tissue Culture" .The term Plant Tissue Culture is commonly used to describe *in vitro* growth and maintenance of plant cell, tissue organs or whole plant on artificially prepared nutritive medium under controlled conditions in a culture room.

Plant Tissue Culture is considered as a noble method to select, multiply and conserve the critical genotypes of medicinal plants by using techniques such as, micropropagation. During the last two decades the techniques of tissue culture have been exploited extensively to study the morphogenetic potential of organs, tissues and cells. Tissue culture can be adopted for

- Species that are difficult to regenerate through conventional breeding methods .The only way to save them is by means of tissue culture.

- The plant population is decreasing due to ever exploitation and hence initial bulk of the stock can be maintained by tissue culture.
- Tissue culture can be applied for genetic improvement of crops.

The various other applications of tissue culture are as follows:

- Clonal propagation
- Conservation through cryopreservation
- Transgenic plants
- Somatic hybridization
- Somaclonal variation
- Artificial seeds production etc

Tissue culture provides an easy and inexpensive way for international exchange of disease free material (Murashige, 1977;Withers, 1980).

Thus considering the importance and feasibility of plant tissue culture technology, in the present investigation two medicinally as well as economically important plants i.e. *Mentha arvensis L.* and *Ammi majus* L. were taken into consideration.

1.3 *Mentha arvensis* (Labiatae / Lamiaceae)

Mentha arvensis is a multipurpose medicinal aromatic herb. A Japanese variety of this plant named *Mentha arvensis* L.sub specie haplocalyx Briq.var piperascens Holmes) has been cultivated in Jammu and Kashmir.

1.3.1 GENERAL ACCOUNT

Mint is a popular and well-known herb growing all over the world and includes many varieties and hybrids. Mint is believed to have come originally from the East and introduced to Europe by way of North Africa and has become naturalized in the *Americas. Mentha arvensis* (English name field mint, corn mint. Hindi, Beng., Mar., Guj. and Tel. –Podina, Pudina KAN.- chetni maragu).Japanese mint is an important variety in Japan

,Taiwan, Brazil, Australia and the USA . Mints are valued in the United States for their essential oils, which had a value of about $ 74 million in 1906 (Green, 1987). The greatest hazard with mint growing is the possibility of rust developing on the foliage. Because of mints sterility, improvement of their tolerance or résistance to pests is not possible using conventional breeding methods. However, variation generated by the use of tissue culture regeneration techniques has resulted in improvement of diverse commercial crops (Resich, 1983).

An alternative for improving mint may involve the use of tissue culture regeneration and the production of somaclonal variants (Larkin and Scowcroft, 1981). Todate, orange mint and peppermint embryos (Van Eck and Kitto, 1990), Japanese mint *(M.arvensis)* have been proliferated *in vitro*, rooted and acclimatized *in vivo*. The genotype dependent regeneration may be due to difference in genetic control of organogenesis by the different mint genotypes (Barocelli et al., 1974).

Mentha arvensis yields and oil; its trade name is Japanese mint oil. On steam distillation the oil is usually distilled from partially dried herbs, leaves and flowering tops give the highest yield. Mint oil is used as a substitute for peppermint oil, which resembles its physico-chemical properties.

In addition to menthol, which is the main constituent, the oil contains methylacetate, menthone and minor amounts of pipertone,α-pinene, furfural, l–limonene, camphene, coryophyllene, d-3 octanol, α–β hexenic acid and other free and esterified fatty acids .The oil distilled from plants grown in Jammu contains 70-80% menthol; dementholized oil has the following composition; Menthol, 44.8; Menthyl acetate, 24.4; menthone ,24.6; and hydrocarbons,6.2%, α-pinene,l-limonene, caryophyllene,cadinene and unidentified sesquiterpene has been identified.

1.3.2 BOTANICAL DESCRIPTION

HABIT: Perennial Herb

ROOT: Tap root

STEM: Erect sometime prostrate, herbaceous, quadrangular or square purplish or green in colour.

LEAVES: Simple, opposite, petiolate, decussate, lanceolate, exstipulate having margins toothed.

INFLORESCENCE: Dichasial or circinate cyme.

FLOWERS: Bisexual, complete, actinomorphic, pentamerous, hypogynous

CALYX: 5 sepals, gamosepalous, persistant, imbricate, bilabiatae (the lobes occasionally obsolete and seemingly 2).

COROLLA: Typically 5 lobed, gamopetalous. The corolla is differentiated into two parts .The lower called the tube and an upper known as the limb. In *Mentha*, two of the upper teeth are united, thus forming an almost regular four lobed corolla.

ANDROCEIUM: Epipetalous, didynamous, usually consists of four stamens the fifth posterior stamens being almost always absent, longitudinal dehiscence.

GYNOECIUM: Ovary superior, 4 lobed, locules 2 or seemingly 4 by intrusion of ovary wall. Placentation basal and derived from axile type the style 1 and gynobasic. Ovules 4, anatropous, erect at the micropyle and facing downwards.

FRUIT: Fruit composed of typically 4 nutlets (rarely pericarp fleshy, distinct cohering in pairs enclosed with persistent calyx).

SEEDS: Seeds are either non endospermic or with very little endosperm.

1.3.3 MEDICINAL IMPORTANCE:

All types of mints have a distinctive flavour and refreshing aroma .The dried leaves and flowering tops of the plant constitute the drug Infusion of its leaves is used in rheumatic pains, aching head, and indigestion. Aerial

parts of the plant are refrigerant, stomachic, carminative, stimulant and diuretic. They possess antispasmodic and emmenagogue properties and are also given to stop vomitting and to treat jaundice. The oil is a valuable antineuralgic.

1.3.4 ECONOMIC IMPORTANCE:

Mint oil is used for flavouring purposes, foods, beverages. Dementholized oil is employed for mouthwash; toothpastes etc.

In India menthol production is non-promising because of the restricted cultivation. Thus, there is need to expand cultivation of this herb in order to ensure round the year availability.

1.4 *Ammi majus* L. (APIACEAE / UMBELLIFERAE)

This family includes 125 genera and 2000 species and has a worldwide distribution particularly in the temperate and subtropical regions (Europe, North Africa, West Central and North Asia). The genus Ammi, a native of Egypt is commonly known as Bishops weed

1.4.1 BOTANICAL DESCRIPTION:

HABIT: Branched annual herb

ROOT: Tap root, Branched

STEM: Erect, Branched, Green Glabrous with large pith that shrinks or dries at maturity with the internode becoming hollow.

LEAF: Alternate, pinnately divided

INFLORESCENCE: Small white terminal umbels.

FLOWER: Bisexual, complete, actinomorphic, polygamous.

CALYX: White in colour, adnate to the ovary and only the 5 lobes distinct and often reduced to teeth, or obsolete enations.

COROLLA: 5 distinct petals, epigynous, often with the apex inflexed. The petals are mostly white.

ANDROCEIUM: 5 stamens, alternate with the petals, Anthers introrse, 2 celled, basifixed, dehiscing longitudinally.

GYNOECIUM: Bicarpellary, syncarpous, ovary inferior. Bilocular with axile placentation, having a single ovule in each locule. Ovules are anatropous and pendulous.

FRUIT: Cremocarp. Fruit is also very characteristic being a schizocarp. Oil tubes(vittae)present in the intercostals spaces (the intervals between the ribs).

1.4.2 MEDICINAL IMPORTANCE:

The Bishops weed produces strongly aromatic seeds. The seeds contain drugs, used in unani system of medicine. The seeds bear ammoidin (Xanthotoxin), ammidin (imperatonin) and majudin (bergaten). All these three compounds are used in the treatment of leucoderma, vitiligo, psoriasis and other dermal dieseas. Bishops weed reputedly helps to treat patchy skin pigmentation of normal skin and testing of antitumour activity.

1.4.3 ECONOMIC IMPORTANCE:

Ammi majus L. is well known for its horticultural importance .It is also used in the manufacture of the suntan lotions in the west and thus has a great export potential.

So, far the work done on this plant is restricted to medicinal and chemical aspects. However, the *in vitro* culture in umbelliferae as a whole is very limited and particularly of *Ammi majus L.* Since, *Ammi majus* L. is a seasonal plant it is difficult to get the material the entire year for manufacturers for the purpose of drug. Keeping in view the medicinal importance of this plant it has been tried to study *in vitro* for its callusing and morphogenic potentials .So that the raw material can be made readily available as and when needed.

1.5 OBJECTIVES OF PRESENT INVESTIGATION

Considering the economic as well as medicinal uses of *Mentha arvensis* and *Ammi majus*. The present work has been conducted with the following objectives:

To investigate the hormonal responses and optimum culture conditions for organogenesis in different explants.

To develop an efficient protocol for raising complete plants *in vitro* and their acclimatization.

An additional factor must be investigated to improve regeneration if tissue culture techniques are to be used for the improvement of commercial medicinal plants.

Furthermore, the protocol standardized in this overall investigation can be used in pharmaceutical industries for the extraction of desired drugs.

REVIEW OF LITERATURE

CHAPTER-2

REVIEW OF LITERATURE.

The present chapter deals about the earlier work done in the field of tissue culture with reference to families Labiatae and Apiaceae in general and *Mentha arvensis* and *Ammi majus* in particular.

The German botanist Haberlandt (1902) was the first who visualized the idea of growing plant cell in artificial nutrient media with the hope of rejuvenating a quiescent cell and triggering it into divisions and growth to from a tissue and eventually regenerate a whole plant. He attempted to grow the pallisade tissue of *Lamium purpureum* in artificial nutrient medium *in vitro* Robbins (1922) and Kotte (1922) further strengthened the idea of totipotency.

Skoog and Miller (1957) studied chemical regulation of growth and organ formation in plant tissue cultured *in vitro* subsequent efforts were directed towards study of nutritional aspects affecting growth of organs and of cell proliferation in *vitro*, to establish continuously growing tissues of a number of plant species capable of being subcultured and maintained for long periods.

2.1 Literature on *Mentha arvensis* and related genera of family (Labiatea).

Based on the literature available on *Mentha arvensis* and its related genera, tissue culture work has been reviewed as given below:

Ono, (1982) reported budding from callus culture of *Mentha arvensis* var. piperascens on medium supplemented with NAA (1 mgl^{-1}) + Kn (1mgl^{-1}).

Rech and Pires (1986) reported rapid multiplication from axillary buds of six *Mentha* species. They observed that nodal segments grown on Murshige and Skoog (MS) medium supplemented with BAP (1.0; 2.0 mgl^{-1}) and Kn (1.0 mgl^{-1}) produce several shoots (15-20 shoots per explant) with

roots. Similarly Pal *et al.* (1985) observed *in vitro* organogenesis and somatic embryogenesis from leaf explants of *Leucosceptrum canum* sm on MS fortified with BA (2,5 mgl^{-1}) alone.

Repcakova *et al.* (1986) obtained shoots from leaf explants of *Mentha piperita* on Linsmaier and Skoog solid (0.8% agar) or Semi Solid (0.4%) agar medium added with BA (0.1,5.0 mgl^{-1}) or Kn (0.1,1.0 mgl^{-1}). Rooting was induced on MS with increased sucrose concentration (6%) and reduced (0.25%) organic N contents.

Mariska *et al.* (1987) cultured stem and stolon explants. Shoot explants cultured on MS medium containing sucrose(30 mgl^{-1}) + group B vitamins + Kn or BAP(5,10 mgl^{-1}) promoted more bud formation than stolon explants. Rhizogenesis was promoted by the addition of NAA at (0.5 mgl^{-1}) to the cytokinin containing medium.

Pierik,(1987) reported that explant source form various species of higher plants has been regenerative. Similarly, Rodov and Davidova (1987) reported the propagation of mint by meristem culture on Lin and Stab medium + IBA (0.1 mgl^{-1}) + GA (0.1 mgl^{-1}). They observed that the propagation rate increased by amending the medium with BAP (0.5 mgl^{-1}) and rooting the medium. In the same year Rajasekaran K. *et al.* observed increased morphogenetic capacity of explants from the basal portion of leaves has been due to higher levels of both IAA and abscissic acid.

Bhaumik and Datta (1988) observed intiation of callus from MS leaf explant of *Mentha arvensis* on MS medium + phenylalanine (100, 500 mgl^{-1}) + High phosphate (170 mgl^{-1}). Similarly, Ravishankar and Venkataraman (1988) reported rapid multiplication from cultured axillary buds of *Mentha piperita* on MS medium supplemented with activated charcoal + IAA + 2,4-D + NAA initiated the formation of multiple shoots which also formed roots when transferred to NAA supplemented medium, whereas, 2,4-D + Kn developed multiple plantlets with a well developed root system.

Geslot *et al*. (1989) studied excision of microcuttings and culturing of microcuttings on MS medium containing IBA and BAP results in *in vitro* multiplication of *Mentha*.

Eck JM Van *et al*.. (1990) reported shoot regeneration from callus of *Mentha piperita* that developed either on mature embryos cultured on MS medium fortified with BAP ($0.5mgl^{-1}$) + NAA ($0.5mgl^{-1}$) or on immature embryos cultured on basal medium containing BAP ($1mgl^{-1}$) + TIBA ($1mgl^{-1}$). Shoots were proliferated, rooted and acclimatized with 100% survival.

Eck JM Van *et al*. (1992) cultured leaf discs from peppermint (*Mentha piperita*), spearmint (*Mentha spicata*) and reported shoot regeneration on media having BAP and coconut water (cw). Orange mint leaf discs regenerated the greatest number of shoots on a basal medium containing 4.4μM BAP enriched with cw at 250 $mllitre^{-1}$ rather than with NAA or TIBA. Peppermint leaf discs regenerated shoots on basal medium having 44.4μM BAP and 250 or 450 ml cw $litre^{-1}$.

Kawabe *et al*.. (1993) cultured adventitious bud pieces of *Mentha arvensis* on a Gamborg B_5 medium fortified with BAP at (0, 0.2, 2 or 4 mgl^{-1}) regeneration of plantlets was observed. Similarly, Bajrovic *et al*. (1993) reported increased callus of *Coleus blumei* and tobacco on a medium having PDP –(1-6 purinyl) -2,5-dimethyl pyrrole (PDP) and Kinetin at concentrations of 0.0, 0.01, 1.0 or 5.0 μM. Whereas, the weight increases in Kinetin treated tobacco callus exceeded those of PDP-treated callus, the reverse trend was found for *Coleus blumei* callus.

Kukreja (1996) reported micropropagation and shoot regeneration from nodal and leaf explants of peppermint (*Mentha piperita* L). He observed that nodal explants cultured on MS medium supplemented with Kn ($2,3\ mgl^{-1}$) + IAA($1mgl^{-1}$), or BAP ($2,3\ mgl^{-1}$) + IAA ($0.25mgl^{-1}$)produced multiple axillary and adventitious shoots which rooted on MS medium containing IAA ($0.25\ mgl^{-1}$).Morphogenetic responses of leaf explants were

growth regulator dependent. Optimal regeneration of shoot buds was observed on MS media supplemented with Kn (3 mgl^{-1}) +IAA (1mgl^{-1}), BAP (2,3 mgl^{-1}) + IAA (1mgl^{-1}), or BAP (2,3 mgl^{-1}) + NAA (0.25 mgl^{-1}).

Bandziuliene and Indrisiunaite (1996) observed micropropagation of mint (*Mentha* spp.) cultured on MS+BAP (2mgl^{-1})+ IBA (1mgl^{-1}). In the same year George and Ravishankar develop modified plant tissue culture media using alternative nitrogen i.e. composite fertilizer "Sulphala" and vitamins i.e. *Spirulina platensis* (vitamin) incorporated in half strength MS medium produced an average of 3 shoots which helps in reducing the costs of micropropagation of *Mentha piperita.*

Berry *et al.* (1997) studied regeneration from leaf disks and petioles in mints was significantly higher on media containing N_6-2- isopentyl adenine (2ip) [isopentyladenine] compared with media containing benzyladenine or Zeatin.

Sajina *et al.* (1997) using shoot tips and nodal explants derived from *in vitro* germinated seedlings of 13 herbal species including *Mentha* and standardized protocols for micropropagation.

Khanuja *et al.* (1998) studied a rapid procedure for isolating somaclones of altered genotypes from an internode explant of *Mentha arvensis* cv. Himalaya. They obtained calluses followed by regeneration of explant on MS medium containing 0.2μg 2,4-D and 7μg cytokinin per ml.

Xue Qi Han *et al.* (1998) cultured young stem segments of peppermint variety 73-8 (*Mentha arvensis* L) and reported somaclonal variation, cell dedifferentiation, plantlet regeneration rate (5%), callus induction (90%) and micropropagation on B_5 medium + 0.1, 1.0 NAA mgl^{-1} +0.4,5.0 BAP mgl^{-1}. In the same year Shasany *et al.* (1998) reported high regenerative nature for shoot regeneration from internodal segments of *Mentha arvensis* excised from *in vitro* raised shoots cultured on MS+ 2μg NAA + 10 μg $BAPml^{-1}$ and 1μg NAA + 5μg $BAPml^{-1}$.

Faure *et al.* (1998) reported *in vitro* shoot organogenesis from leaf disks of peppermint (*Mentha piperita*) and spearmint (*Mentha spicata*) grown on MS+ 300μM mannitol + 2.0 μM 6-benzyl-adenine + 2.0 μM indole 3 butyric acid and rooting was obtained on MS+0.5μM NAA + 9.0 μM BA + 0.5 μM thidiazuron.

Abou Mandour and Binder (1998) reported effect of exogenous growth regulators on plant regenerants from tissue culture of *Mentha spicata*. Best callus induction was observed on Abou Mandour (AM) medium with Kn, 2,4-D, IAA ($2mgl^{-1}$ each). Leaf and shoot explants were the best source for compact and nodulated callus.

Ruseva (1999) obtained *in vitro* culture of mint (*Mentha piperita*) by microcuttings. High multiplication rates was observed an Linsmaier and Skoog (LS) medium added with BAP ($0.5\ mgl^{-1}$) + NMPE (natural mint plant extract) on LS+IAA ($1mgl^{-1}$) + NMPE single shoot regeneration was induced.

Li Xia *et al.* (1999) reported highest frequency of meristemoids and morphogenetic callus from leaf explants of *Mentha spicata* L. cultured on MS+ TDZ ($4mgl^{-1}$) + 25% vol/vol coconut water (cw). Bud and shoot development required removal of both TDZ and cw from the medium. To facilitate shoot elongation shoot propagules were transferred to MS + NAA ($0.01mgl^{-1}$). Individual shoots (1cm tall) were transferred onto the same medium which results in rhizogenesis.

Shahzad and Siddiqui (2000) reported *in vitro* organogenesis in *Ocimum sanctum* from nodal explants cultured on MS + 2,4-D ($2mgl^{-1}$) proved best for the induction of organogenic callus; multiple shoots differentiated when grown on MS+BA ($5mgl^{-1}$) + NAA ($0.2mgl^{-1}$) + glutamic acid ($5mlg^{-1}$) Early callus induction followed by profuse rhizogenesis was observed when cultured on MS+NAA ($5mgl^{-1}$)+BA ($0.5mgl^{-1}$). The microshoots rooted well on MS+NAA ($1.5mgl^{-1}$) and the plantlet successfully acclimatized in soil.

Liu *et al.* (2000) reported regeneration of *Salvia sclarea* via organogenesis from immature zygotic embryo cotyledons (IZEC) on the culturing medium enriched with 9.05 μM 2,4-D. The organogenic tissue were then proliferated on media containing IAA+BA shoots regenerated from both proliferated tissue and IZEC propagated in the presence of IAA or αNAA, BA and GA_3 roots were induced from regenerated shoots on the media having low conc. of IAA, IBA (0.98μM). The regenerated plants were transferred to soil for further growth.

2.2 Literature on *Ammi majus* and related genera of family Apiaceae.

Based on the literature available on *Ammi majus* and related genera of this family tissue culture work has been reviewed here as given below:

Sehgal (1972) described *in vitro* induction of polyembryony from excised ovaries of *Ammi majus* cultured on MS+beef extract + CH/YE.

Grewal *et al.* (1976) reported plant regeneration from hypocotyl cultures of *Ammi majus* L. When cultured on MS medium containing IAA at 2ppm.

Thorpe and Patel (1984) reported cytokinin or combination of cytokinin and auxin is required for clonal propagation.

Gosal *et al.*, (1991) cultured mature stem segments of *Apium graveolens* and reported micropropagation through somatic embryogenesis on MS medium. The micropropagated plantlets were grown, successfully in soil.

Laxmi *et al.* (1991) studied *Daucus carota* callus maintained on Millers' modified medium + NAA (2p.p.m) + Kn (0.5 ppm) callus were transferred to medium having NAA (0, 0.5, 1.0, 1.5 or 2.0 p.p.m) and MBC (Methyl benzimidazolyl carbomate) [Carbendazin] 0,2,4,8 or 10 ppm. Nearly, all combinations of MBC and NAA induced root and shoot formation in the 2^{nd} subculture with the number of shoots produced increases MBC concentration.

Song *et al*. (1991) studied the effect of plant growth regulators on embryogenic callus induction and adventive embryogenesis from leaf and stem segments of *Foeniculum vulgare* Gaertner cultured on MS + 2, 4-D ($0.01mgl^{-1}$) + BA ($0.01mgl^{-1}$).

Song *et al*.. (1991) also studied the effect of complex addenda and sucrose on adventive embryogenesis from leaf and stem segments of *Foeniculum vulgare* Gaertner cultured on MS with high conc. of malt extract (500 mgl^{-1}) + sucrose ($50mgl^{-1}$) increases the frequency of embryogenesis.

Song *et al*. (1991) studied the effect of plant growth regulators on plant regeneration from adventive embryos. Somatic embryos cultured in hormone free medium results in 77% regeneration of normal plantlets than in media having 2, 4-D (63%), benzyladenine (67%) or Kinetin (47%) alone. The best combination of growth regulators was BA ($0.01mgl^{-1}$) + 2,4-D ($0.01mgl^{-1}$).

Theiler and Kagi (1992) reported cloning *in vitro* and somatic embryogenesis from axillary and apical buds of *Foeniculum vulgare* Mill. (fennel) of 'Zefa Fino' and 'Zefa Tardo' when cultured on modified MS media. Somatic embryo formation was genotype as well as tissue dependent, being particularly good with nodal segment explants.

Schiavo *et al*. (1992) studied cells of *Daucus* grown in suspension culture in Gamborg's B_5 medium with 2, 4-D ($0.5mgl^{-1}$) and BA ($0.25mgl^{-1}$) and resuspended in medium containing BAP ($0.25mgl^{-1}$) + 2,4-D (0.1-3.0 mgl^{-1}) such that embryogenesis was induced.

Michalczuck *et al*.. (1992) reported regulation of indole 3-acetic acid in biosynthetic pathways of *Daucus* cell cultures. In the presence of 2,4-D accumulation of tryptophan derived IAA is promoted during callus proliferation that is not active during somatic embryo formation.

Smith and Krikorian (1992) cultured embryogenic cultures initiated from seedling hypocotyl on nutrient medium having NAA or 2,4-D in cultivated *Daucus carota* (CV-scarlet Nantes) concluded that it was the pH of the culture medium and not the presence or absence of an auxin or the N (source (s)) that permitted or prevented cell elongation in the embryogenic culture tested.

Nissen *et al.* (1992) reported stimulation of somatic embryogenesis in *Daucus* by ethylene. It is the endogenous ethylene levels which were suboptimal for somatic embryogenesis in suspension culture.

Kataeva *et al.* (1993) obtained three clones from individual seedlings of *Coriandrum sativum* and micropropagated alternately on MS+ Kn or MS + Kn + IAA.

Jager *et al.* (1993) obtained callus culture of *Thapsia garganica* on a medium containing 2, 4-D ($1mgl^{-1}$). When transferred to media without 2,4-D produce somatic embryos that developed into plantlets.

Sushma *et al.* (1993) raised callus from explants of shoot meristem on revised MS medium with 1x10-5 M 2,4-D, 3% sucrose and 0.8% agar.

Jay *et al.* (1994) revealed that somatic embryogenesis was affected by the pH changes in *Daucus carota* L. Embryo production was greatest when the pH of the hormone free medium was maintained at 4.3.

Madhumati *et al.* (1995) obtained callus of cotyledonary leaves of *Ammi majus* cultured on MS + α IAA + Kn + CH and reported that addition of adenine to the medium induced shoot formation and plantlet were produced on MS + IBA + glutamine.

Purohit *et al.* (1995) investigate that the callus obtained from cotyledonary leaves in *Ammi majus* grown on MS+IAA ($2mgl^{-1}$)+Kn ($5mgl^{-1}$) + CH ($1000\ mgl^{-1}$) differentiated shoot buds on a medium additionally enriched with adenine. Plantlets (3.5cm tall) resulted when transfer of

shoots to MS+IBA+ glutamine containing media. These plantlets flowered *in vitro*.

Hunault and Maatar (1995) studied the effect of GA_3 on somatic embryogenesis from petiole fragments excised from micropropagated *Foeniculum vulgare* plantlets. Petiole explant cultured on induction medium devoid of growth regulators allows embryo development. The addition of filter sterilized GA_3 to the embryo development medium increased the number of embryogenic explants. No positive effect was observed when GA_3 was added to the micropropagation medium of the mother plantlets.

Maatar and Hunault (1997) When cultured petiole explants of *Foeniculum vulgare* Mill. reported somatic embryogenesis on medium having 2,4-D and MCPA (0.5, 1.0 mgl^{-1}) which were more effective than IAA or NAA. However, a high auxin (2,4-D) concentration addition of cytokinin (Kn or BAP) to the medium inhibited the induction of embryogenesis.

Gupta *et al.* (2001) reported that TDZ (0.5 and 0.1 mgl^{-1}) induced 30% regeneration in *Cuminum cyminum* L. 8 shoots per regenerated shoots could be multiplied on 0.5 mgl^{-1} Kn which rooted on 1.0 mgl^{-1} IAA.

CHAPTER-3

MATERIALS AND METHODS

3.1. EXPERIMENTAL MATERIALS:

The present investigation deals with two economically as well as medicinally important plants i.e. *Mentha arvensis* L. and *Ammi majus* L. The explant used includes namely leaves, nodal segments and shoot tips (0.5-1.5cm). Were obtained from young healthy plants growing in natural habitat, at Botanical Garden of Botany Department, A.M.U, Aligarh.

3.2 EXPERIMENTAL METHODOLOGY:

3.2.1 COMPOSITION OF MURASHIGE & SKOOGS (MS) (1962): MEDIUM

Growth and regeneration of plant tissues *in vitro* are largely governed by the composition of the culture media. Nutritional requirements for optimal growth of a tissue *in vitro* may vary with the species. A number of different media have been devised but the most suitable medium to induce organogenesis and regeneration in cultured tissues is the Murashige & Skoogs (MS) medium, which contains the desired salt composition. In the present investigation a basal medium proposed by Murashige and Skoog (MS) (1962) has been used. The different constituents of MS medium along with their concentration are given in Table-1.

Table-1: COMPOSITION OF MURASHIGE AND SKOOG'S MEDIUM (MS) (1962)

MAJOR SALTS	mgl^{-1} (lx)	mgl^{-1} (10x)	gml^{-1} (10x)
$NH_4 NO_3$	1650	16500	16.50
KNO_3	1900	19000	19.00
$CaCl_2. 2H_2O$	440	4400	4.40
$MgSO_4. 7H_2O$	370	3700	3.70

KH_2PO_4	170	1700	1.70
Na_2EDTA	31.3	313	0.313
$FeSO_4.7H_2O$	27.8	278	0.278

MINOR SALTS	mgl^{-1} (lx)	mgl^{-1} (10x)	gml^{-1} (10x)
H_3B0_3	6.2	62	0.062
$MnSO_4.\ 4H_2O$	22.3	223	0.223
$ZnSO_4.7H_2O$	8.6	86	0.086
KI	0.83	8.3	0.0083
$Na_2MoO_4.\ 2H_2O$	0.25	2.5	0.0025
$CuSO_4.5H_2O$	0.025	0.25	0.00025
$CoCl_2.\ 6H_2O$	0.025	0.25	0.00025

VITAMINS	mgl^{-1} (lx)	mgl^{-1}(10x)	gml^{-1} (10x)
Myo-inositol	100	1000	1.000
Nicotinic acid	0.5	5	0.005
Pyridoxine HCl	0.15	5	0.005
Thiamine. HCl	0.1	1	0.001
Glycine	2.0	20	0.02

3.2.2 GROWTH HORMONES: In addition to the medium, it is essential to add one or more growth substances, such as auxins and cytokinins to support good growth of tissue.

3.2.3 AUXINS: The auxins used in the present investigation are Indole 3-acetic acid (IAA), Indole 3-butyric acid (IBA), Naphthalene acetic acid (NAA), and Dichlorophenoxy acetic acid 2,4-D. In tissue cultures auxins have been used for cell division and root differentiation. Auxins are dissolved in few drops of absolute alcohol.

3.2.4 CYTOKININS: The cytokinins used in the present study are Benzyl amino acid (BAP), and 6-Furfuryl amino purine (Kn). In tissue

culture media, cytokinins are incorporated mainly for cell division and differentiation of adventitious shoots from callus and organs. Cytokinins are dissolved in 0.1N NaOH and made to volume by adding DDW. This stock solution is used in different concentrations.

3.2.5 **CARBON SOURCE**: Plant cells and tissues in the culture media lack autotrophic ability and therefore incorporate external carbon as source of energy. The most preferred carbon source in plant tissue culture is sucrose at a concentration of 3% in the medium necessary for various metabolic activities. It is required for differentiation of xylem and phloem elements in cultured cells (Aloni, 1980)

3.2.6 **GELLING AGENT:** Agar a polysaccharide obtained form seaweeds is most commonly used in the medium to form a firm gel at 1% concentration. It provides a wide surface area to growing tissue in the static condition.

3.3.0 **PREPARATION OF MEDIA**: All hormones, vitamins and constituents given in (Table-1) were dissolved separately in double distilled water to form a stock solution and were stored in a refrigerator at 10-15^{0}C.

$FeSO_4$ and Na_2EDTA are dissolved separately and added to the medium lastly to avoid precipitation of the salts.

To prepare basic culture medium, appropriate quantities of MS medium, vitamins and hormones were taken from stock solutions and then double distilled water was added to make final volume. Then 3% sucrose and 1% agar were mixed and medium was cleared in hot water bath. The pH of the medium was adjusted to 5.8 by using 0.1N NaOH or 0.1N HCl. About 20ml of the medium was dispensed in a culture tube. All the culture tubes were labelled and plugged with non absorbent cotton.

3.4.0 STERILISATION: The aseptic techniques are necessary for maintaining aseptic environment in order to avoid contamination. The technique involves.

3.4.1 STERILISATION OF NUTRIENT MEDIA: The nutrient medium and distilled water used for washing purposes were commonly sterilized by autoclaving at 15 lbs/inch^2 and 121^{0}C for 15-20 minutes. After autoclaving the slants were made by placing culture tubes overnight in an inclined position.

3.4.2 STERILIZATION OF GLASSWARES AND INSTRUMENTS: The glasswares to be used were wrapped in aluminium foil or butter paper and sterilized by autoclaving while the instruments such as forceps, scalpels etc. which are made up of stainless steel were surface sterilized by dipping in rectified spirit and flamed on spirit lamp before each inoculation.

3.4.3 STERILIZATION OF PLANT MATERIALS: The explants i.e nodal segments, leaves and shoot tips were collected in a flask containing water. The material was washed thoroughly under tap water for 30 minutes then it is taken in inoculation room. The material was treated with 5% teepol for 5 minutes with continuos shaking of flask followed by rapid washing 3-4 times with sterilised DDW. Thereafter, the explants were surface sterilized for 2-5 minutes depending upon the material. These were subsequently washed with sterilized double distilled water 4-5 times to remove the traces of sterilant. At last suitable explants are selected and were replaced in sterilised petriplates for inoculation in culture tubes.

3.4.4 STERILIZATION OF LAMINAR FLOW BENCH: The laminar flow bench was sterilised by uv light for about 1-2 hours before the work was started. The table top of the laminar flow is wiped with 70% alcohol.

3.5.0 INOCULATION AND INCUBATION: Inoculation of explant in the culture tube was done in aseptic environmental conditons either in incoculation room or inside the laminar flow bench. The leaf and stem explants were cultured after cutting into small pieces of suitable size. All the explants whether it is shoot tip, nodal segment, or leaf should be placed at the centre of slant and the tube should be plugged quickly after inoculation.

After inoculation the culture tubes were incubated under controlled environmental conditions of culture room. Usually, air conditioners are used to maintain the temperature around 25± 2°C, 16 hours light and 8 hours dark illumination was provided by fluorescent tubes of 500 lux. The humidity was maintained between 50-60%.

3.6.0 SUBCULTURING: All the cultures were maintained by subculturing on fresh medium on a regular interval of 3-4 weeks.

3.7.0 HARDENING PROCEDURE/ACCLIMATIZATION:

Regenerated plantlets with well differentiated shoot and roots were first thoroughly washed with water in order to remove the adhering medium. Plantlets were then transferred to pots containing sterilized mixture of soil and soilrite (3:1) covered with polybags, watered and maintained at high humidity maintained. They were kept in growth chambers at 25±2°C for 15 days. After that plants were transferred to earthen pots for acclimatization in natural conditions.

3.8.0 PRECAUTIONS:

(i) Always keep away the hand moistened with alcohol from the spirit lamp so dry the alcohol first.

(ii) Exposure to uv light builds up a high concentration of ozone gas inside the closed chamber. In case, working inside an inoculation chamber it is healthy to enter the chamber only 15-30 minutes after switching off the uv lamp.

(iii) Do not dip hot instruments in alcohol and don't use hot instrument for cutting or holding the plant material.

(iv) Don't heat the neck of the culture tubes excessively.

OBSERVATIONS

CHAPTER 4

OBSERVATIONS

4.1 *Mentha arvensis* L.

4.1.1 LEAF EXPLANTS:

Leaf explants of *Mentha arvensis* were cultured on MS basal medium supplemented with different auxins and cytokinins in variable concentrations. Observation were taken every week in order to study associated changes in colour, appearance, and texture of the callus, alongwith organogenic response of the explant both directly and indirectly. **(Table 2, Hist. 1)**

Leaf explants inoculated on MS basal medium without growth regulator (control) showed direct numerous shoots with brownish white friable callus in nature after 10 days of inoculation and after 19 days of inoculation size of callus increased considerably **(Plate 1, Fig. A).**

When the explant was cultured on MS basal medium supplemented with IBA ($2mgl^{-1}$) it showed formation of dark brown compact callus from the whole surface of leaf after 7 days of inoculation. Later on the size of callus increased and exhibited late indirect induction of rhizogenesis i.e. after an incubation period of 28 days.**(Plate 1, Fig .B)**

Tender leaf explants when inoculated on MS medium with NAA ($2mgl^{-1}$) exhibited swelling in whole lamina within 3 days of inoculation and after further incubation of 10 days callus was brown in colour, compact in nature and on around 20 days indirect numerous whitish hairy roots were formed from the callus which increased considerably in length on subsequent incubation **(Plate 1,Fig .C).**

The explant cultured on MS basal medium enriched with BAP ($2mgl^{-1}$) exhibited creamish white compact callus within 6 days of inoculation and after further incubation of 15 days indirect shoot was formed. **(Plate1, Fig .D)**

The explant when cultured on MS basal medium augmented with BAP ($2mgl^{-1}$) + IBA ($2mgl^{-1}$) exhibited slight swelling after 3 days of inoculation. Direct single shoot bud from the midrib region was observed after 6 days of inoculation. Rhizogenesis was not observed from the shoot let on the same medium even after 20 days of incubation.**(Plate 2, Fig A).**Further shoots were cut and sub cultured on BAP($2mgl^{-1}$) + NAA ($1mgl^{-1}$) this resulted in brown compact callogenesis and indirect caulogenesis and rhizogenesis was observed after 13 days of transfer. **(Plate 2, Fig B).**

Leaf as an explant cultured on MS medium fortified with BAP($2mgl^{-1}$) + IAA ($1mgl^{-1}$) showed formation of small white compact callus from explant after 4 days of inoculation and within 13 days of incubation few shootlets emerge from the callus which remained as such showing no signs of rhizogenesis on subsequent incubation **(Plate 2, Fig C).**

The leaf explant when inoculated on MS basal medium enriched with BAP ($2mgl^{-1}$) + 2,4-D ($1mgl^{-1}$) exhibited formation of white compact callus from the entire surface of explant after 6 days of inoculation, no organogenic response was observed upto 18 days of incubation **(Plate 2, Fig .D).** Further more explant augmented on MS basal medium fortified with Kn($.5mgl^{-1}$) + NAA ($5mgl^{-1}$) in combination showed white compact callus formation from explant after 8 days of incubation, growth of callus continued and indirect rhizogenesis was observed on 15^{th} days of inoculation. **(Plate 2.Fig .E)**

The explant when inoculated on MS basal medium fortified with Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$) exhibited brown friable callusing from the whole surface of explant after a weeks' period of incubation and after 13 days the size of callus increased considerably followed by formation of numerous indirect roots. **(Plate 3, Fig .A).** After 3 weeks callus was subcultured on different medium i.e. MS basal medium enriched with Kn ($.5mgl^{-1}$) + IBA ($5mgl^{-1}$) exhibited indirect caulogenesis callogenesis and rhizogenesis. **(Plate 3, Fig .B)**

When the explant was cultured on MS basal medium augmented with Kn ($2mgl^{-1}$) + 2,4-D ($1mgl^{-1}$), it exhibited formation of white coloured compact callus from the margins of explants after 8 days of inoculation and on further incubation of 25 days no organogenic response was observed. **(Plate 3, Fig.C).** Callus when further subcultured on MS basal medium augmented with Kn ($0.5mgl^{-1}$) + IBA ($5mgl^{-1}$) showed indirect caulogenesis, rhizogenesis and callogenesis (i.e. brown compact callus in nature within 22 days of transfer **(Plate 3, Fig. D)**.

Leaf explants when cultured on MS basal medium fortified with Kn ($3mgl^{-1}$) + NAA ($.5mgl^{-1}$) showed white compact callus after 8 days of inoculation and 2 roots originating were appeared from midrib region of explant after further incubation of 24 days. **(Plate 4, Fig .A)**

The explant when cultured on MS basal medium augmented with Kn ($5mgl^{-1}$) + NAA ($.5mgl^{-1}$) exhibited few number of shoots after 10 days of inoculation .On prolonged incubation of 24 days numerous shoots appeared from the explant **(Plate 4, Fig .B).**

Leaf as an explant when cultured on 1/2 MS basal medium augmented with Kn ($5mgl^{-1}$) + NAA ($.5mgl^{-1}$) in combination showed swelling and expansion in lamina within 6 days of inoculation which turned into compact white callus after 40 days of incubation direct organogenic response i.e. direct multiple shoots and roots were observed from the explant **(Plate 4, Fig. C).**

Leaf explant cultured on MS basal medium enriched with Kn ($2mgl^{-1}$) + BAP ($2mgl^{-1}$) + IAA ($.5mgl^{-1}$) in combination exhibited small white compact callus from the margins of the explant within 6 days of inoculation. On incubation of 18 days numerous shoots appeared from the petiolar region of the explant. **(Plate 4, Fig. D).**

4.1.2 SHOOT TIP EXPLANTS:

For callus induction and organogensis the sterilized shoot tip explants (1.5-2.0 cm) were taken form the mature plant and were cultured on MS basal medium supplemented with various growth hormones singly as well as in combinations **(Table 3, Hist. 2 and 3)**.

The shoot tip explant when cultured on MS basal medium without any growth regulator (control) showed elongation of explant on 6^{th} day of inoculation attaining a height of 4 cm after 26 days of inoculation **(Plate 5, Fig.A).**

The explant when cultured on MS basal medium augmented with IBA ($2mgl^{-1}$) showed development of dark brown compact callus from the lower portion of the explant after 9 days of inoculation, on further incubation for 20 days indirect multiplication of shoots and emergence of numerous indirect roots was observed **(Plate 5, Fig.B)**. The same explant when cultured on medium with double the initial concentration of IBA (i.e $4mgl^{-1}$) results only in elongation of apical bud on 5^{th} day of inoculation and after an incubation period of 9 days the height of shoot was 6 cm **(Plate 5, Fig.C)**.

The explant when inoculated on MS basal medium fortified with NAA ($2mgl^{-1}$) showed development of dark brown compact callus form the cut end of explant after 6 days of inoculation. Later on large number of indirect roots and multiple shoots appeared within 18 days of incubation period **(Plate 5, Fig.D)**. The same explant when inoculated on medium having double the initial concentration of NAA (i.e. $4mgl^{-1}$) exhibited dark brown compact callus from the entire surface of the explant on 8^{th} day of inoculation. On further incubation for a period of 30 days, elongation of apical bud and large number of indirect hairy roots developed **(Plate 5, Fig. E).**

When the explant was cultured on MS basal medium fortified with BAP ($2mgl^{-1}$), it resulted in proliferation of explant within 9 days of

inoculation the size of the explant was 5cm. It remained the same upto 28th day of inoculation. **(Plate 6, Fig. A)**.

Shoot tip explant cultured on MS basal medium enriched with IAA ($2mgl^{-1}$) showed initiation of direct multiple shoots as well as roots from the portion of the explant which was in contact with the medium after 8 days of inoculation. However, the multiplication of shoots was at a faster rate which was examined in 32 days old culture. **(Plate 6, Fig. B)**.

The explant when inoculated on MS basal medium fortified with IAA ($4mgl^{-1}$) i.e double the intial conc. of IAA ($2mgl^{-1}$) showed elongation of single shoot after 6 days of inoculation. The elongated shoot attain a height of 9cm and no further growth, variation and rhizogenesis was observed upto 25 days of inoculation on the same medium **(Plate 6, Fig. C)**.

Shoot tip explants cultured on MS basal medium enriched with Kn ($2mgl^{-1}$) showed small white compact callus from the whole surface of explant within 9 days of inoculation. On further incubation of 28 days large number of indirect shoots developed from the upper portion of the cut end. Earlier shoot developed showed increase in its length however no rhizogenesis was observed within an incubation period of 40 days **(Plate 6, Fig. D)**.

The explant when inoculated on MS basal medium augmented with combination of Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$) exhibited intiation of shoots after 4 days of inoculation, caulogenesis started from the cut end of the explant. On subsequent incubation shoot proliferates without showing any sign of rhizogenesis within an incubation period of 27 days **(Plate7, Fig.A)**. Shoot tips were subcultureed on same medium which resulted in initiation of indirect rhizogenesis and formation of light brown compact callus within 40 days of culture **(Plate 7, Fig. B)**.

When the explant was cultured on MS basal medium fortified with Kn ($2mgl^{-1}$) + IBA ($1mgl^{-1}$) it exhibited direct shoot emergence after 6 days of

inoculation. No further growth and variation was noticed upto 18 days of inoculation on the same medium.**(Plate 7, Fig.C).** Shoot tip explant when cultured on another medium containing IBA ($1.5mgl^{-1}$) for rhizogenesis. Indirect rhizogenesis, elongation of shoot and light brown almost friable callus was also observed within 18 days of culture **(Plate 7, Fig. D)**.

The explant when inoculated on MS basal medium enriched with Kn ($2mgl^{-1}$) + NAA ($1mgl^{-1}$) exhibited direct elongation of apical bud with emergence of new shoots from the cut end within 11 days of inoculation. On subsequent incubation the growth of the shoot buds was slow without showing any sign of rhizogenesis after 44 days of inoculation **(Plate 8, Fig. A)**.

The explant was cultured on MS basal medium enriched with combination of Kn ($5mgl^{-1}$) + NAA ($0.5mgl^{-1}$). It showed direct shoot emergence after 4 days of inoculation. Few more shoot buds were initiated directly from the shoot tip explant within 13 days of inoculation. The previously induced shoot elongated rapidly and attained its height of about 4cm within 28 days of inoculation**(Plate 8, Fig. B)**.

Shoot tip explant cultured on MS basal medium fortified with Kn ($5mgl^{-1}$) + NAA($1mgl^{-1}$) exhibited multiple shoot proliferation and white small compact callus from the portion of explant in contact with medium after 8 days of inoculation. However, multiplication of shoots was at faster rate no rhizogenesis was observed after 30 days of inoculation
(Plate 8, Fig. C).

The explant when inoculated on MS basal medium enriched with Kn ($5mgl^{-1}$) + IAA ($0.5mgl^{-1}$) results in elongation of shoot and multiplication of shoots within 4 days of inoculation no rhizogenesis was observed within 24 days of inoculation. Subsequent subculturing on different medium i.e. IBA ($1.5mgl^{-1}$) resulted in indirect rhizogenesis, almost brown friable callus within 17 days of subculture and the previously induced shoot elongated rapidly and attained its height of about 9.5 cm **(Plate 8, Figs.D and E)**.

When the explant was cultured on MS basal medium enriched with BAP ($2mgl^{-1}$) + NAA ($0.5mgl^{-1}$) it resulted in slight swelling of the explant within 4 days of inoculation emergence of shoot buds occurred and on subsequent incubation the growth of the shoot buds was slower without any sign of rhizogenesis after 23 days of inoculation **(Plate 9, Fig. A)**.

The explant when inoculated on MS basal medium augmented with BAP ($2mgl^{-1}$)+IAA ($1mgl^{-1}$) resulted in direct multiple proliferation of shoots and aerial roots from the shootlets within 11 days of inoculation. The shoots attained height of 10 cm within 45 days of inoculation **(Plate 9, Fig. B)**.

Shoot tips cultured on MS basal fortified with BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$) exhibited direct multiple shoots and aerial roots from the explant within 15 days of inoculation. The first formed shootlet attained a height of 9.5 cm within 43 days of inoculation **(Plate 9, Fig. C)**.

Shoot tips cultured on MS basal medium supplemented with BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$) in combination exhibited direct multiple shoots within 6 days of inoculation. On subsequent subculturing the growth of the shootlets was slower (4cm) in size without any sign of rhizogenesis within 23 days of inoculation **(Plate 9, Fig. D)**.

Shoot tip as an explant inoculated on MS basal medium supplemented together with BAP ($5mgl^{-1}$) + NAA ($0.2mgl^{-1}$) showed direct multiple shoot emergence after 7 days of inoculation. The elongated shoots not only increase in height but also their number increases however no rhizogenesis was observed within an incubation period of 28 days **(Plate 10, Fig. A)**.

Shoot tip as an explant cultured on MS basal medium enriched with BAP ($2mgl^{-1}$) + Kn ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$) exhibited direct multiproliferation of basal shoots from the portion of explant which was in contact with the medium on 8^{th} day of inoculation on subsequent incubation the height and

number of shoots increase however no rhizogenesis was observed within an incubation period of 30 days **(Plate 10, Fig. B)**. These shoot tips were cultured on NAA ($2mgl^{-1}$) resulting in intiation of indirect rhizogenesis accompanied by brown compact callus at the cut end of shoot noticed on 18^{th} day of subculture **(Plate 10, Fig. C)**.

Explant enriched with MS basal medium fortified with BAP ($2mgl^{-1}$) + NAA ($1.5mgl^{-1}$) resulted in direct multiplication of shoots and rhizogenesis after 8^{th} day of inoculation. The increase in the number of shoots was more as compared to number of roots within an incubation period of 34 days **(Plate 10, Fig. D)**.

ACCLIMATIZATION OF PLANTLETS:

Plantlets with well differentiated shoots and roots were transferred to a plastic pot having sterile mixture of soil and soilrite in 3:1 ratio **(Plate 10, Fig. E)** covered with polythene bags to maintain high humidity. They were kept in a growth chamber 25± 2^0C for 15 days and then transferred to earthen pots containing pure garden soil **(Plate, 10 Fig. F)**. Plantlets were growing well *in vivo* and reared till maturity. An average of 80% survival rate was recorded.

4.1.3 NODAL EXPLANTS

Nodal explants of *Mentha arvensis* were cultured on MS basal medium supplemented with different auxins and cytokinins in variable concentrations. Observations were recorded every week in order to study associated changes in colour, appearance, and texture of the callus along with organogenic response of the explant both directly and indirectly. **(Table 4, Hist. 4)**

The explant inoculated on MS basal medium without growth regulators (control) showed formation of direct single white root within 4 days of inoculation and no sign of caulogenesis were observed on subsequent 25 days of incubation **(Plate 11, Fig .A)**

The explants when cultured on MS basal medium augmented with NAA ($2mgl^{-1}$) exhibited slight swelling after 3 days of inoculation. Direct single shoot from the nodal region was observed after 6 days of inoculation rhizogenesis and callogenesis was not observed from the shootlet on the same medium even after 24 days of inoculation **(Plate 11. Fig .B).**

The explants cultured on MS basal medium fortified with IAA ($2mgl^{-1}$) started direct caulogenesis from the portion of explant that was in contact with the nutrient medium after 5 days of inoculation. Growth of shoot was rapid and continuous upto 20 weeks of inoculation. Further no rhizogenesis or callogenesis was exhibited even after 40 days of transfer. **(Plate 11, Fig .C)**

Nodal segment cultured on MS basal medium enriched with IAA ($4mgl^{-1}$) showed initiation of direct multiple shoots and roots from the portion of the explant in contact with the medium, after 7 days of inoculation. However, the multiplication of shoot buds was not at a faster rate as compared to rhizogenesis after 28 days of inoculation **(Plate 11, Fig .D).**

The Nodal segment cultured on MS basal medium fortified with Kn ($2mgl^{-1}$) exhibited white compact callus initiation after 10 days of inoculation .The growth of callus was very slow, no organogenesis, was observed upto 40 days of inoculation and the growth of callus was stunted and showed no further increase in mass even after 40 days of inoculation **(Plate 11, Fig .E).**

When the explant was cultured on MS basal medium supplemented with BAP ($2mgl^{-1}$) it showed white compact callus and initiation of indirect multiple shoots from the portion of explant in contact with the medium, after 10 days of inoculation. However, the multiplication rate of shoot buds was faster after 42 days of inoculation.**(Plate 11, Fig .F).**

Nodal segment cultured on MS basal medium augmented with Kn ($.5mgl^{-1}$) + IBA ($5mgl^{-1}$) showed initiation of white compact callus after 6

days of inoculation. However, after 25 days of incubation on the same medium the size of callus increases but sign of organogenesis was not observed after 32 days of transfer. **(Plate 12,Fig.A).**

Explants cultured on MS basal medium enriched with Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$) exhibited direct multiple shoots near the cut end of explant after 5 days of inoculation. Although shoots proliferated slowly after 22 day of transfer on same medium but no visible signs of rhizogenesis or callogenesis was observed. **(Plate 12,Fig.B).**

Nodal explants cultured on MS basal medium augmented with Kn ($2mgl^{-1}$) + 2,4-D ($1mgl^{-1}$) showed slight emergence of direct multiple shoot bud formation from the portion of explant which was in contact with the medium after 5 days of inoculation shoots which were observed they failed to show proliferation on the same medium, and no signs of rhizogenesis and caulogenesis were observed even after 20 days of incubation **(Plate 12,Fig .C).**

Nodal segment cultured on MS basal medium fortified with Kn ($2mgl^{-1}$) + NAA ($1mgl^{-1}$) showed emergence of direct multiple shoots from the portion of explant which was in contact with the medium after 4 days of inoculation .On prolonged incubation of 17 days of inoculation the size of shoots increases slowly but no visible sign of callogenesis and rhizogenesis was examined **(Plate 12, Fig .D).**

Nodal segment cultured on MS basal medium enriched with Kn ($2mgl^{-1}$) + IAA ($1mgl^{-1}$) develop direct multiple shoots from the portion of explant which was near to the cut end of the explant after 5 days of inoculation. Shoots which were observed, failed to show rhizogenesis or callogenesis from the explants even after 18 days of incubation on the same medium **(Plate 12, Fig .E).**

Explants cultured on MS basal medium augmented with Kn($2mgl^{-1}$) + IBA ($1mgl^{-1}$) exhibited emergence of direct multiple shoots from the portion

of the explant which was in contact with the medium after 3 days of inoculation on subsequent incubation period of 20 days callogenesis and the initiation of rhizogenesis failed to occur **(Plate 12, Fig. F)**

Nodal segment cultured on MS basal medium fortified with Kn ($2mgl^{-1}$) + BAP ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$) showed initiation of direct caulogenesis within 8 days of inoculation. Later on size of the shoot bud increases attaining a height of (5 cm) on the same medium and no signs of rhizogenesis and or callogenesis were observed even after 32 days of incubation **(Plate 12, Fig.G).** When subcultered on different media i.e. IBA ($1.5mgl^{-1}$), it resulted in initiation of large number of direct roots and one direct shoot appeared from the explant after an incubation period of 17 days **(Plate 12, Fig. H).**

Nodal explant when cultured on MS basal medium fortified with BAP ($2mgl^{-1}$) + 2,4-D ($1mgl^{-1}$) showed shoot induction after 4 days of inoculation. Growth was found to be moderate on the same medium and no rhizogenesis or callogenesis was observed even after 12 days of inoculation **(Plate 13, Fig. A).**

Explants when cultured on MS basal medium supplemented with BAP ($2mgl^{-1}$) +NAA ($1mgl^{-1}$) showed formation of white compact callus from the node after 9 days of inoculation. The initiation of direct caulogenesis was observed from the upper surface of the callus and the callus size remained as such and no rhizogenesis was observed within 36 days of incubation **(Plate 13, Fig. B).**

Explant when inoculated on MS basal medium enriched with BAP ($2mgl^{-1}$) + IAA ($.5mgl^{-1}$) exhibited dull white nodular callus from the whole surface of explant 9 days after inoculation on further incubation of 36 days on the same medium the size of callus increases and no caulogenesis or rhizogenesis was observed. **(Plate 13, Fig. C)**

Explants when cultured on MS basal medium augmented with BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$) showed emergence of direct multiple shoots from the portion of explant which was in contact with the medium after days of inoculation .A good number of shoots increased in height (about 5-6cm) these were formed within 23 days of inoculation. **(Plate 13, Fig. D).**

Nodal explants cultured on MS basal medium enriched with BAP ($3mgl^{-1}$) + IAA ($1.5smgl^{-1}$) abruptly showed direct multiple shoot emergence and direct rhizogenesis from the upper cut end of explant after 8 days of inoculation. After 5 weeks the growth of directly induced shoots were found to be comparatively more than root. Previous shoots showed rapid increase in length however, growth of other shoots continued. **(Plate 13, Fig.E)**

4.1.4 ACCLIMATIZATION OF PLANTLETS

Plantlets with well differentiated shoots and roots were transferred to a plastic pot containing sterile mixture of soil and soilrite in 3:1 ratio **(Plate 13, Fig .F)** covered with polythene bags to maintain high humidity. They were kept in a growth chamber $25\pm2^{0}C$ for 15 days and then transferred to earthen pots containing pure garden soil **(Plate 13 Fig .G)** Plantlets grew well *in vivo* and reared till maturity. An average of 60% of survival rate was recorded.

4.2 Ammi majus L

4.2.1 LEAF EXPLANT

Small leaf segments used as explants were cultured on MS basal medium supplemented with various growth regulators either singly or in various combinations **(Table 5, HIST.5).**

The leaf explants cultured on MS basal medium without growth regulators (control) showed expansion of lamina and explants remained green in colour for 11 days. Later on, it showed no further response *in vitro* upto 30 days of incubation **(Plate 14, Fig. A)**

Leaf explant cultured on MS basal medium fortified with NAA ($2mgl^{-1}$) exhibited direct rhizogenesis from the surface of the explant after 10 days of inoculation which continued slowly upto 40 days of inoculation .**(Plate 14, Fig. B)**

When the explant was cultured on MS basal medium, augmented with NAA ($2mgl^{-1}$) in combination with BAP ($3mgl^{-1}$) it showed swelling followed by callogenesis in the whole lamina after 8 days of inoculation. Growth of callus was fast and after 32 days of inoculation considerable amount of callus mass was obtained. Callus was creamish green in colour and friable in nature. **(Plate 14, Fig .C)**

The explant as a leaf portion cultured on MS basal medium supplemented with Kn ($0.5mgl^{-1}$)+IBA ($5mgl^{-1}$) exhibited initiation of callus after 8 days of inoculation. There was a gradual increase in callus growth and large amount of callus mass was obtained after further incubation of 32 days. Callus was dark yellow in colour and nodular in nature **(Plate 14, Fig.D)**

Leaf explant cultured on MS basal medium fortified with Kn ($3mgl^{-1}$)+NAA ($1.5mgl^{-1}$) showed callus formation from the entire surface of the explant after 6 days of inoculation. Growth of callus was very slow and within 24 days of incubation callus was found to be creamish in colour and nodular in nature **(Plate 14, Fig.E)**

The leaf explant when cultured on half strength of MS basal medium augmented with Kn($5\ mgl^{-1}$) + NAA($0.5mgl^{-1}$) induced callogenesis after 10 days of inoculation. Initiation of callus along the margins of leaf lamina was noticed .Callus was yellow in colour and nodular in nature. **(Plate 15, Fig. A).** After 41 days of incubation subculturing was done on MS+BAP ($2mgl^{-1}$) there was gradual increase in callus growth, large amount of nodular callus was observed .The colour of callus changed from yellow to

brown and concomitantly emergence of indirect shoot bud initiation was observed within 30 days of incubation. (**Plate 15, Fig. B)**

Leaf explant when cultured on MS basal medium enriched with BAP ($3mgl^{-1}$) in combination with IAA ($2mgl^{-1}$) showed initiation of callus after 10 days of inoculation. Growth of callus was slow on 40 days of incubation. Callus was found to be whitish green in colour and compact in nature. (**Plate 15,Fig. C).**

The explant when cultured on MS basal medium augmented with BAP ($2mgl^{-1}$)+Kn ($2mgl^{-1}$)+IAA ($0.5mgl^{-1}$) exhibited callogenesis from the whole surface of the explant after 8 days of inoculation. Growth of callus was moderate. Major portion of the callus was yellow with few green patches, increase in friability was noticed after 32 days of inoculation. **(Plate 15, Fig. D)**

4.2.2 NODAL SEGMENTS:

Nodal segments of *Ammi majus* were cultured on MS basal medium enriched with various plant growth regulators either singly or in various combinaitons **(Table 6, Hist. 6)**

Nodal segments of *Ammi majus* cultured on MS basal medium showed no response *in vitro* upto 30 days of inoculation Later on, the explant started drying.

Nodal segments cultured on MS basal medium augmented with IBA ($2mgl^{-1}$) alone showed elongation of shoot buds present in axil of nodal region of explants followed by formation of inflorescence **(Plate 16, Fig .A)**

When the nodal segments were cultured on MS basal medium enriched with IAA ($1.5mg^{-1}$) alone, they exhibited caulogenesis after 5 days of inoculation. Later on *in vitro* inflorescence emerged out from the apical bud of the explant within 15 days of inoculation. Although the growth of shoot buds was slow but a continuous multiplication of shoots was

observed. A continuous increment in the number of shoots (6 in number) was observed within 19 days of inoculation **(Plate 16, Fig. B)**

The nodal portion of the explant when inoculated on MS basal medium fortified with IAA ($2mgl^{-1}$) showed callogenesis and buds present in the axils of nodal reign of explants elongated after 5 days of inoculation. *In vitro* inflorescence and the growth of callus was found to be slow till 13 days of incubation.Callus was yellow in colour and somewhat friable in nature. On subsequent incubation direct root initiation was observed from nodal region facing the medium downwards. Which was in contact with the medium, and concomitantly *in vitro* inflorescence also developed from the proliferated shoot on 22^{nd} day of inoculation **(Plate 16, Fig. C)**.

Nodal segments cultured on MS basal medium supplemented with IAA ($3mgl^{-1}$) exhibited callogenesis after 17 days of inoculation. Callus was smooth, brown and friable in nature. But the growth of callus was rapid with few roots induced directly from the callus after 30 days of inoculation **(Plate 16, Fig. D)**.

Nodal segments cultured on MS basal medium fortified with IAA ($4mgl^{-1}$) showed elongation of apical bud after 5 days of inoculation. Direct rhizogenesis and *in vitro* inflorescence was induced from the apical shoot on 22^{nd} day of inoculation. **(Plate 16, Fig. E)**

Nodal explants cultured on MS basal medium augmented with a combination of BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$) showed greenish compact callus initiation from the cut end of explant after 4 days of inoculation and on further incubation of 15 days the shoots elongated directly but slowly and concomitantly resulted in the initiation of *in vitro* inflorescence. Shoots were cut and subcultured on medium containing IBA ($2mgl^{-1}$) but shoots did not show any sign of rhizogenesis after 40 days of its transfer.
(Plate 17, Fig .A)

Nodal segments cultured on MS basal medium enriched with BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$) showed elongation of single shoot along with *in vitro* inflorescence after 4 days of inoculation little bit of callusing from the nodal portion of explant was observed after 17 days of inoculation . Callus was light green in colour and friable in nature. Shoots were isolated and subcultured on MS basal medium fortified with BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$) elongation of shoot was observed but no rhizogenesis was observed upto 35 days of transfer. **(Plate 17, Fig.B)**

Nodal explants cultured on MS basal medium enriched with Kn ($5mgl^{-1}$)+IAA ($.5mgl^{-1}$) showed emergence of abrupt multiple shoot buds after 15 days of inoculation, directly from the nodal region of the explant, which was facing the medium. Shoot buds were isolated and subcultured on the same medium to induce rhizogenesis. No such remarkable effect of growth hormones was observed in this case even after 40 days of transfer. **(Plate 17, Fig.C)**

Nodal segments inoculated on MS basal medium supplemented with IAA ($2mgl^{-1}$) + Kn($1.5\ mgl^{-1}$)exhibited formation of single shoot alongwith 6 leaves after one week of inoculation and within 35 days of incubation rhizogenesis was also observed which later on undergoes elongation **(Plate 17,Figs. D, E).**

4.2.3 ACCLIMATIZATION OF PLANTLETS

In vitro formed shoots with root system were transferred to plastic pots containing soil and soilrite mixture in the ratio of 3:1.The plastic pots were then covered with plastic bags to maintain the high humidity (90%) and placed in growth chambers. After about 2 weeks the plants were then transferred to earthen pot containing garden soil and reared to maturity. **(Plate 17, Fig.F).**

TABLE-2 Effect of growth hormones on Leaf explant of *Mentha arvensis* L.

MS+ Hormones (mgl^{-1})	CALLUS INDUCTION	ORGANOGENESIS			
	Colour and Nature	Caulogenesis		Rhizogenesis	
		Direct	Indirect	Direct	Indirect
CONTROL	Brownish white, Friable	++	--	--	--
IBA (2)	Dark brownish ,compact	--	--	--	+++
NAA (2)	Brownish, compact	--	--	--	++
BAP (2)	Creamish white, compact	--	++	--	--
BAP (2)+IBA (2)	--	+	--	--	--
BAP (2) + NAA (1)	Brown, compact	--	+	--	++
BAP (2) + IAA (1)	White, compact	--	++	--	--
BAP (2) + 2,4-D (1)	White, compact	--	--	--	--
Kn (0.5) + NAA (5)	White, compact	--	--	--	+++
Kn (1) + NAA (2)	Brown, Friable	--	--	--	+++
Kn (0.5) + IBA (5)	Brown, Friable	--	+	--	++
Kn (2) + 2,4-D (1)	White, compact	--	--	--	--
Kn (0.5) + IBA (5)	Brown, compact	--	+	--	+++
Kn (3) + NAA (0.5)	White, compact	--	--	+	--
Kn (5) + NAA (0.5)	--	+++	--	--	--
1/2 MS+ Kn (5) + NAA (0.5)	White, compact	+++	--	+++	--
Kn (2)+ BAP (2) + IAA (0.5)	White, compact	--	+++	--	--

-- No response ; + Poor ; ++ Moderate ; +++ Best.

Table-3 Effect of growth hormones on Shoot tip explants of *Mentha arvensis* L.

MS+ Hormones (mgl^{-1})	CALLUS INDUCTION	ORGANOGENESIS			
	Colour and Nature	Caulogenesis		Rhizogenesis	
		Direct	Indirect	Direct	Indirect
CONTROL	--	+	--	--	--
IBA (2)	Dark brown, compact	--	+++	--	+++
IBA (4)	--	+	--	--	--
NAA (2)	Dark brown, compact	+++	--	+++	--
NAA (4)	Dark brown, compact	+	--	--	+++
BAP (2)	--	+	--	--	--
IAA (2)	--	+++	--	+++	--
IAA (4)	--	+	--	--	--
Kn (2)	White, compact	--	+++	--	--
Kn (1) + NAA (2)	--	+	--	--	--
Kn (1) + NAA (2)	Light brown compact	+	--	--	++
Kn (2) + IBA (1)	--	++	--	--	--
IBA (1.5)	Light brown compact	+	--	--	++
Kn (2) + NAA (1)	--	++	--	--	--
Kn (5) + NAA (0.5)	--	++	--	--	--
Kn (5)+NAA (1)	White, compact	+++	--	--	--
Kn (5) IAA (0.5)	--	++	--	--	--
IBA (1.5)	Brown, fraible	+	--	--	++
BAP (2) + NAA (0.5)	--	++	--	--	--
BAP (2) + IAA (1)	--	+++	--	++	--
BAP (2) + NAA (1)	--	++	--	+	--
BAP (2) + IAA (1)	--	++	--	--	--
BAP (5) + NAA (.2)	--	+++	--	--	--
BAP (2) + Kn (2)+IAA (0.5)	--	+++	--	--	--
NAA (2)	Brown, compact	+	--	--	++
BAP (2) + NAA (1.5)	-	++	--	++	--

-- No response ; + Poor ; ++ Moderate ; +++ Best.

TABLE-4 Effect of growth hormones on Nodal explant of *Mentha arvensis* L.

MS+ Hormones (mgl^{-1})	CALLUS INDUCTION	ORGANOGENESIS			
	Colour and Nature	Caulogenesis		Rhizogenesis	
		Direct	Indirect	Direct	Indirect
CONTROL	--	--	--	+	--
NAA($2mgl^{-1}$)	--	+	--	--	--
IAA ($2mgl^{-1}$)	--	++	--	--	--
IAA ($4mgl^{-1}$)	--	++	--	+++	--
Kn ($2mgl^{-1}$)	White, compact	--	--	--	--
BAP ($2mgl^{-1}$)	White, compact	--	--	--	--
Kn ($.5mgl^{-1}$) + IBA ($5mgl^{-1}$)	White, compact	--	--	--	--
Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$)	--	++	--	--	--
Kn ($2mgl^{-1}$) + 2,4- D ($1mgl^{-1}$)	--	+	--	--	--
Kn ($2mgl^{-1}$) + NAA ($1mgl^{-1}$)	--	++	--	--	--
Kn ($2mgl^{-1}$) + IAA ($1mgl^{-1}$)	--	++	--	--	--
Kn ($2mgl^{-1}$) + IBA($1mgl^{-1}$)	--	++	--	--	--
Kn ($2mgl^{-1}$) + BAP($2mgl^{-1}$) + IAA ($.5mgl^{-1}$)	--	+	--	--	--
IBA($1.5mgl^{-1}$)	--	+	--	+++	--
BAP($2mgl^{-1}$) +2,4-D($1mgl^{-1}$)	--	+	--	--	--
BAP($2mgl^{-1}$) +NAA($1mgl^{-1}$)	White, compact	+++	--	--	--
BAP($2mgl^{-1}$) + IAA ($.5mgl^{-1}$)	Dull white, nodular	--	--	--	--
BAP($2mgl^{-1}$) +IAA($1mgl^{-1}$)	--	+++	--	--	--
BAP($3mgl^{-1}$) +IAA($1.5mgl^{-1}$)	--	+++	--	++	--

-- No response ; + Poor ; ++ Moderate ; +++Best.

Table 5. Effect of growth hormones on leaf explant of *Ammi majus L.*

MS+ Hormones (mgl^{-1})	CALLUS INDUCTION	ORGANOGENESIS			
	Colour and Nature	Caulogenesis		Rhizogenesis	
		Direct	Indirect	Direct	Indirect
CONTROL	--	--	--	--	--
NAA (2)	--	--	--	+++	--
BAP (2)	Brownish, nodular	--	+	--	--
NAA (2) + BAP(3)	Creamish green, friable	--	--	--	--
Kn (0.5) + IBA (5)	Dark yellow, nodular	--	--	--	--
Kn (3) + NAA (1.5)	Creamish, nodular	--	--	--	--
Kn (5) + NAA (0.5)	Yellow, nodular	--	--	--	--
BAP (3) + IAA (2)	White-green, compact	--	--	--	--
BAP (2) + Kn (2) + IAA (0.5)	Yellow-green, friable	--	--	--	--

-- No response ; + Poor ; ++ Moderate ; +++ Best .

Table 6: Effect of growth hormones on Nodal explant of *Ammi majus* L.

MS+ Hormones (mgl^{-1})	CALLUS INDUCTION	ORGANOGENESIS				
	Colour and Nature	Caulogenesis		Rhizogenesis		
		Direct	Indirect	Direct	Indirect	Inflorescence
CONTROL	--	--	--	--	--	--
IBA (2)	--	+	--	--	--	+++
IAA (1.5)	--	+++	--	--	--	++
IAA (2)	Yellow, Friable	+	--	++	--	++
IAA (3)	Brown, Friable	--	--	--	++	--
IAA (4)	--	+	+	+	--	++
BAP (2) + NAA (1)	Greenish, compact	+	--	--	--	+
BAP (2) + IAA (1)	Light green, Friable	+	--	--	--	+
Kn (5) + IAA (0.5)	--	++	--	--	--	--
IAA (2) + Kn (1.5)	--	+	--	++	--	--

-- No response ; + Poor ; ++ Moderate ; +++ Best .

Source of Explant

Source of Explant

Explanation of Plate 1

(Figs. A-D)

Fig.A) 19 days old culture showing formation of numerous shoots and brownish white friable callus from leaf explant cultured on MS + without hormone.

Fig.B) 28 days old culture showing dark brown compact callus with direct rhizogenesis from leaf explant cultured on MS+IBA ($2mgl^{-1}$).

Fig.C) 20 days old culture showing brownish compact callus with numerous indirect roots from leaf explant cultured on MS+NAA($2mgl^{-1}$).

Fig.D) 15 days old culture showing indirect caulogenesis and creamish white compact callus from leaf explant cultured on MS+BAP($2mgl^{-1}$).

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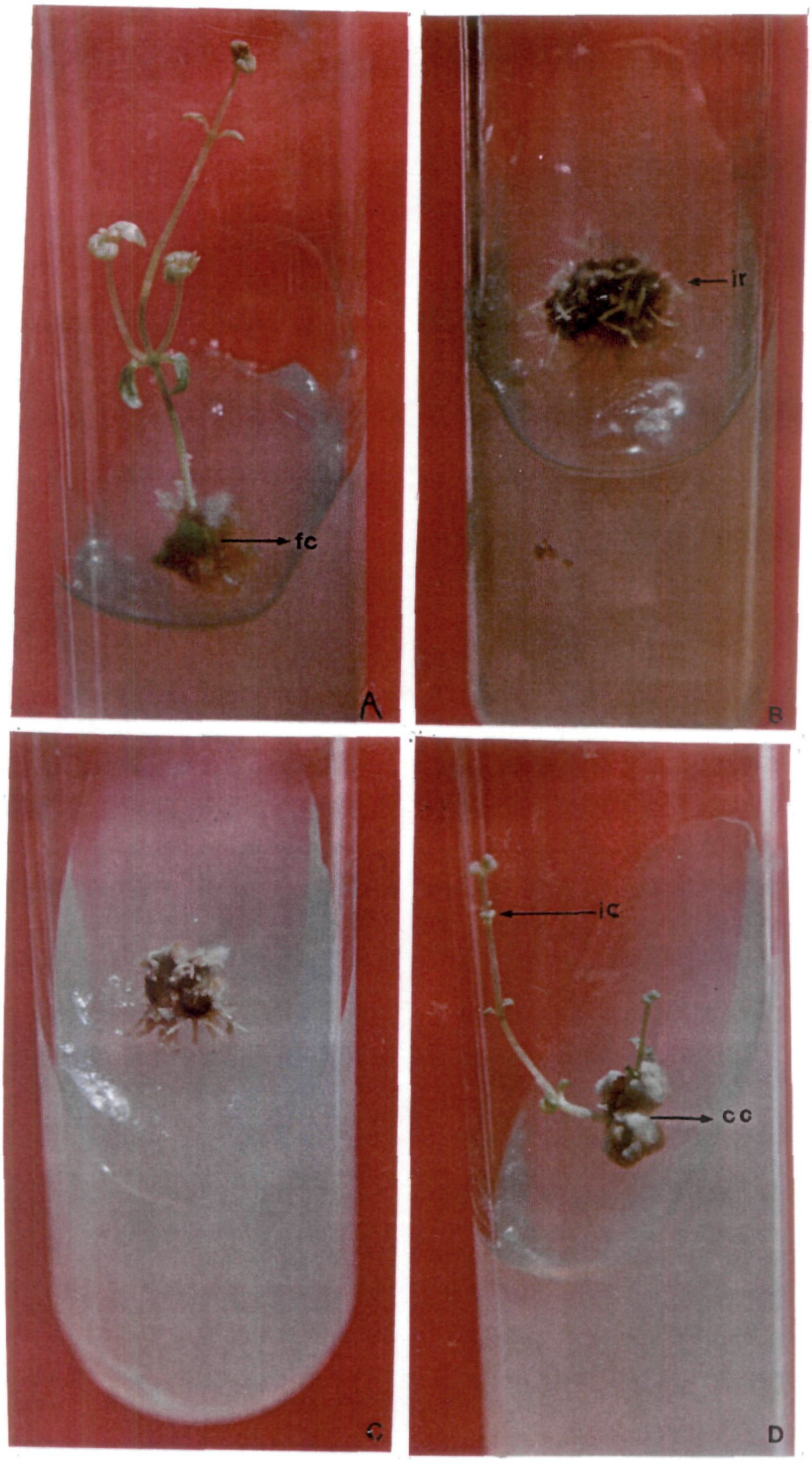
fc-friable callus ; ir-indirect rhizogenesis ;

ic- indirect caulogenesis ; cc- compact callus.

Plate 1

Figs A–D



Explanation of Plate-2

(Figs. A-E)

Fig A) 20 days old culture showing elongation from leaf explant cultured on MS + BAP ($2mgl^{-1}$) + IBA ($2mgl^{-1}$).

FigB) 13 days old culture showing callogenesis and indirect caulogenesis and rhizogenesis from leaf explant cultured on MS + BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$).

Fig C) 13 days old culture showing small white compact callus as well as direct caulogenesis from leaf explant cultured on MS+BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$).

Fig D) 18 days old culture showing white brown compact callus from leaf explant culturedon MS + BAP ($2mgl^{-1}$) + 2,4-D ($1mgl^{-1}$).

Fig E) 15 days old culture showing white compact callus and indirect rhizogenesis from leaf explant cultured on MS + Kn ($.5mgl^{-1}$) + NAA ($5mgl^{-1}$).

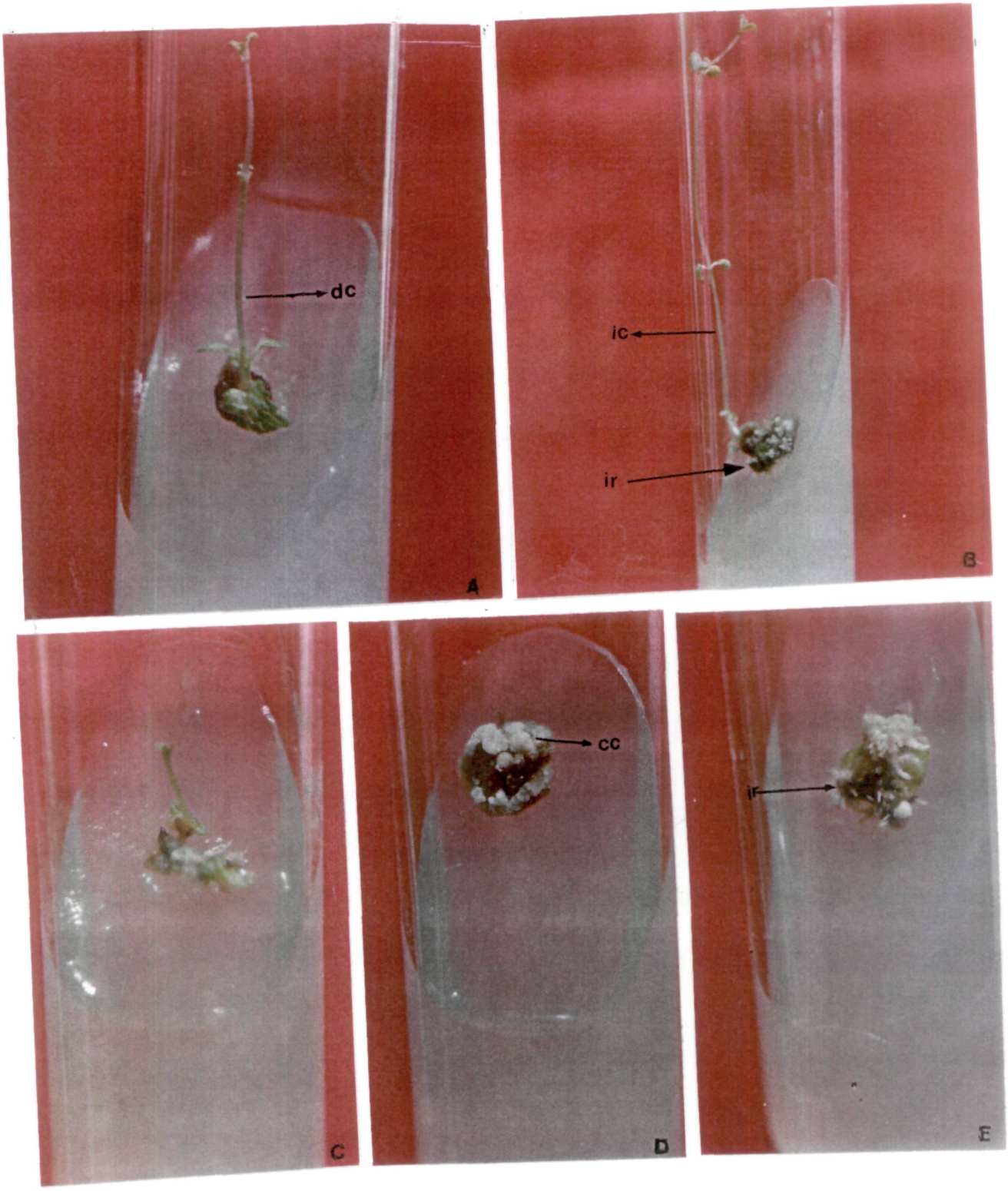
---

dc- direct caulogenesis ; ic indirect caulogenesis ;

cc- compact callus ; ir- indirect rhizogenesis.

Plate 2

Figs A – E



Explanation of Plate 3

(Figs. A-D)

Fig A) 13 days old culture showing brownish friable callus and indirect rhizogenesis from leaf explant cultured on MS + Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$).

Fig B) 21 days old culture showing indirect rhizogenesis, caulogenesis and callogensis when leaf explant cultured on MS + Kn ($.5mgl^{-1}$) + IBA ($5mgl^{-1}$).

Fig C) 25 days old culture showing white compact callus along with the margins of leaf explant inoculated on MS+ Kn ($2mgl^{-1}$)+2,4-D ($1mgl^{-1}$).

Fig D) 22 days old culture exhibiting indirect caulogensis, rhizogenesis and callogenesis when leaf explant cultured on MS + Kn ($.5mgl^{-1}$) + IBA ($5mgl^{-1}$).

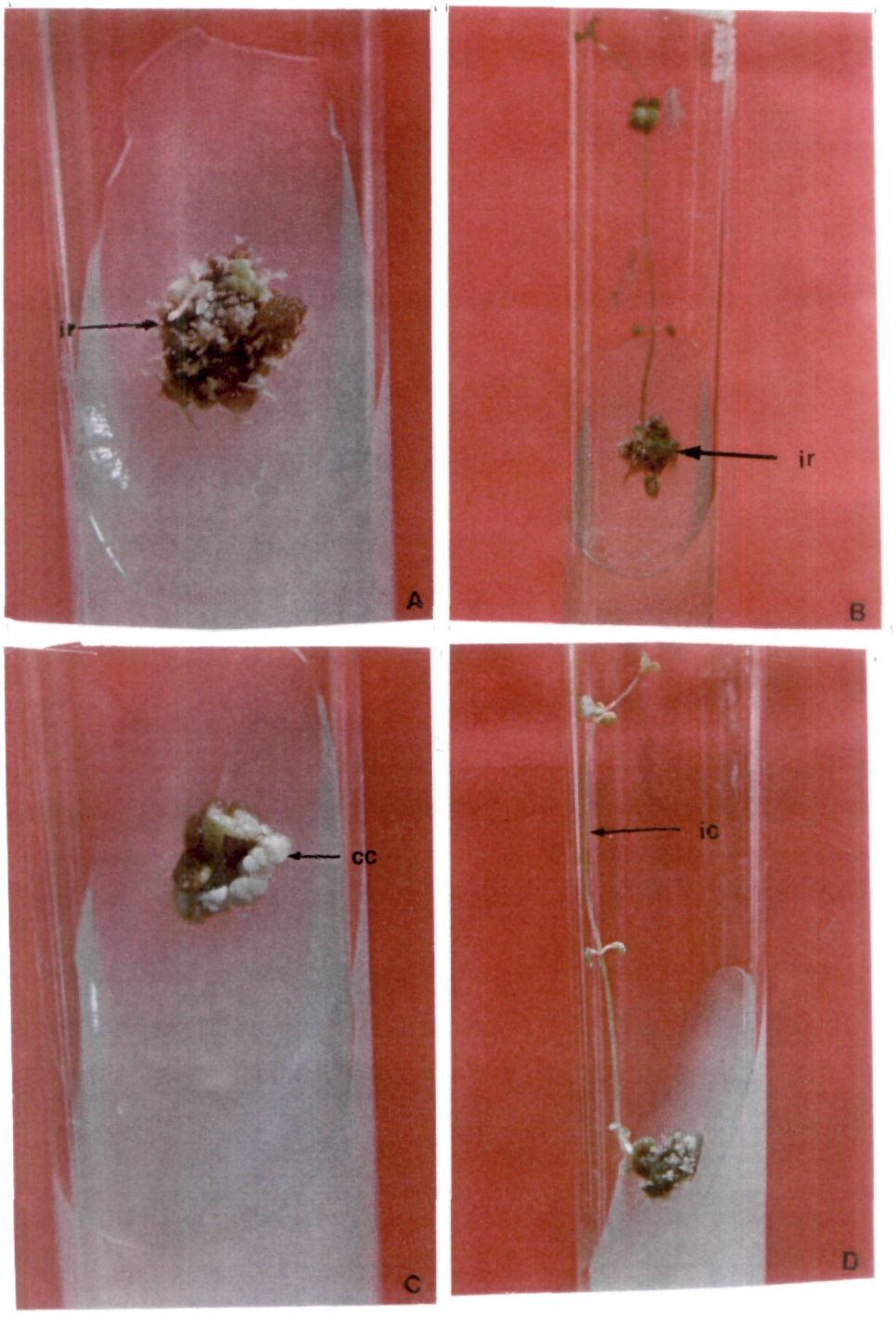
---

ir- indirect rhizogenesis ; cc- compact callus;

ic- indirect caulogenesis.

Plate 3

Figs A – D



Explanation of Plate 4

(Figs. A-D)

Fig .A) 24 days old culture exhibiting white compact callus and induction of 1-2 roots when leaf explant cultured on MS + Kn ($3mgl^{-1}$) + NAA ($.5mgl^{-1}$).

Fig B) 24 days old culture showing direct caulogenesis when leaf explant cultured on MS + Kn ($5mgl^{-1}$) + NAA ($.5mgl^{-1}$).

Fig.C) 40 days old culture showing white compact callus and direct organogenesis when leaf explant cultured on ½ MS + Kn ($5mgl^{-1}$) + NAA ($.5mgl^{-1}$)

Fig D) Petiolar region of leaf explant when cultured on MS+Kn ($2mgl^{-1}$) +BAP ($2mgl^{-1}$)+IAA ($5mgl^{-1}$) exhibited white compact callus and direct caulogenesis.

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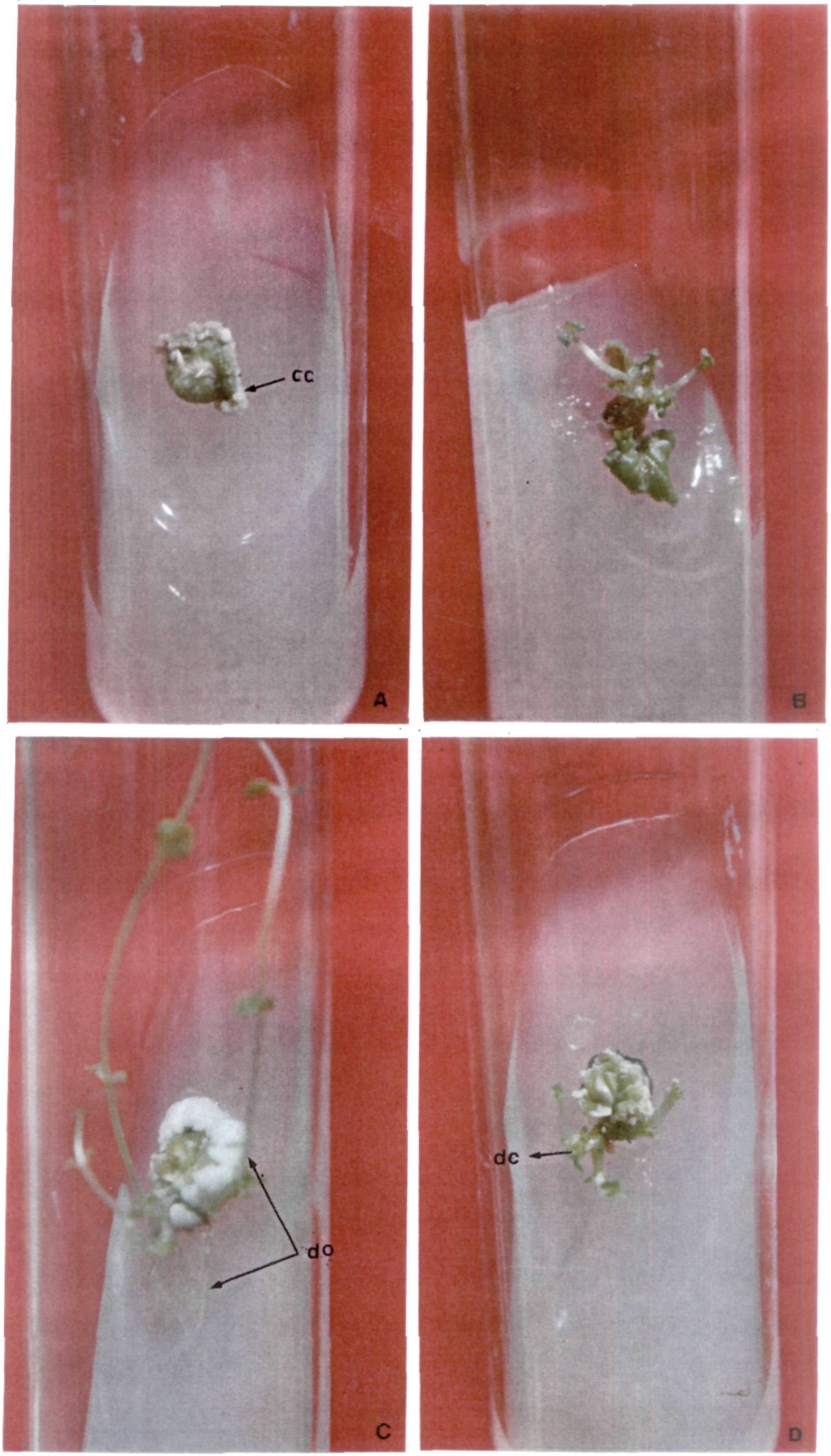
cc-compact callus; dr-direct caulogenesis;

do-direct organogenesis.

Plate 4

Figs A – D



Explanation of Plate 5

(Figs. A-E)

Fig. A) 26 days old culture showing elongation of shoot tip explant cultured on MS + without hormones.

Fig. B) 20 days old culture showing dark brown compact callus, indirect multiplication of shoots and roots from shoot tip explant cultured on MS+ IBA ($2mgl^{-1}$)

Fig. C) 19 days old culture showing elongation of apical bud when cultured on MS+IBA ($4mgl^{-1}$)

Fig. D) 18 days old culture showing dark brown compact callus, indirect multiple roots and shoots obtained from shoot tip explant cultured on MS+NAA ($2mgl^{-1}$)

Fig. E) 30 days old culture showing dark brown compact callus, indirect rhizogenesis and elongation of apical bud when shoot tip explant cultured on MS+NAA ($4mgl^{-1}$).

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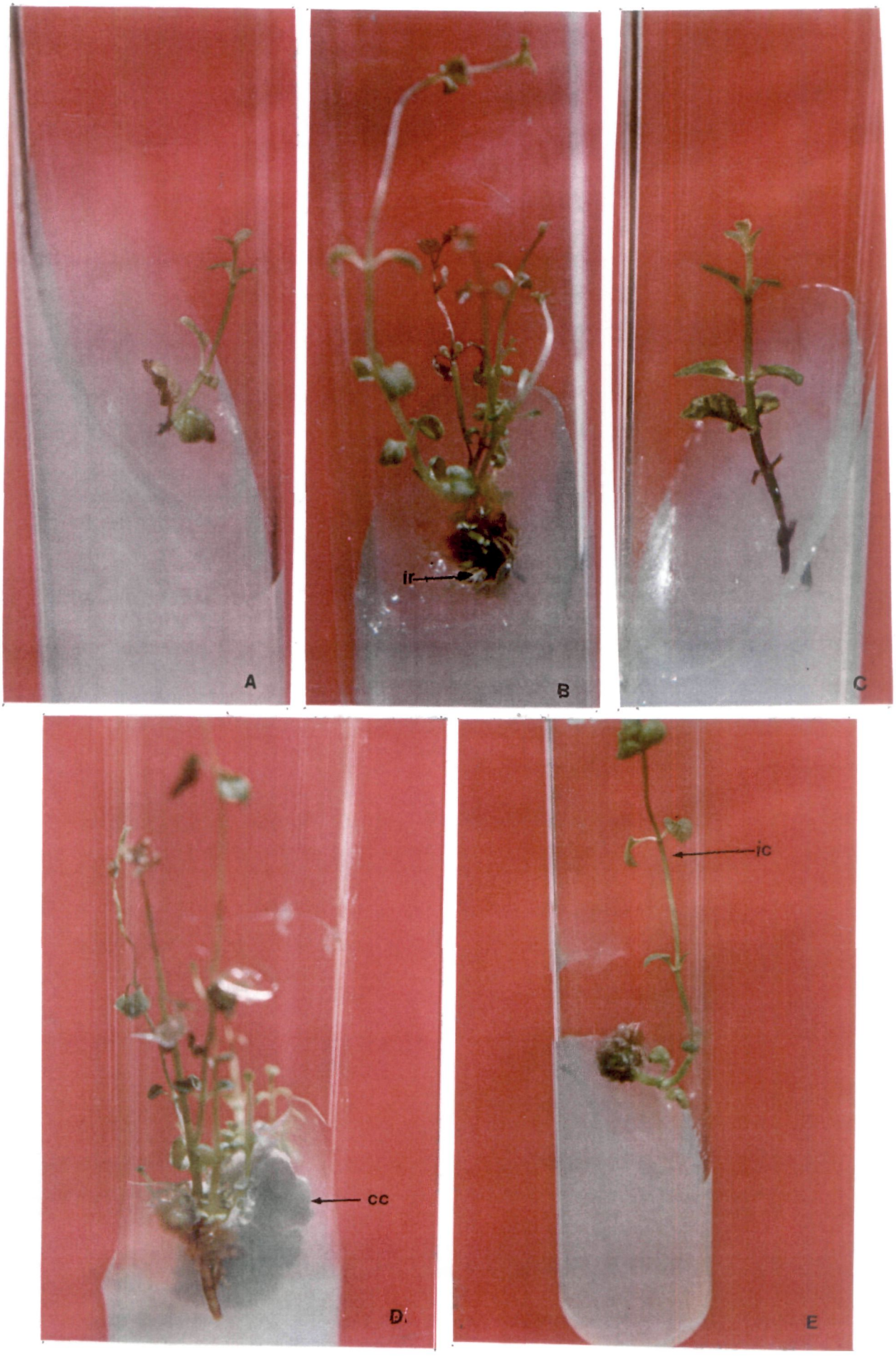
cc- compact callus; ir- indirect rhizogenesis;
ic- indirect caulogenesis.

Plate 5

Figs A – E



Explanation of Plate 6

(Figs. A-D)

Fig A) 28 days old culture showing elongation of shoot bud obtained on MS + BAP ($2mgl^{-1}$).

Fig B) 32 days old culture showing direct enhanced caulogenesis and rhizogenesis when shoot tip explant cultured on MS+IAA ($2mgl^{-1}$).

Fig C) 25 days old culture showing elongation of apical bud cultured on MS + IAA ($4mgl^{-1}$).

Fig D) 40 days old culture showing white compact callus and indirect caulogenesis from shoot tip explant cultured on MS + Kn ($2mgl^{-1}$).

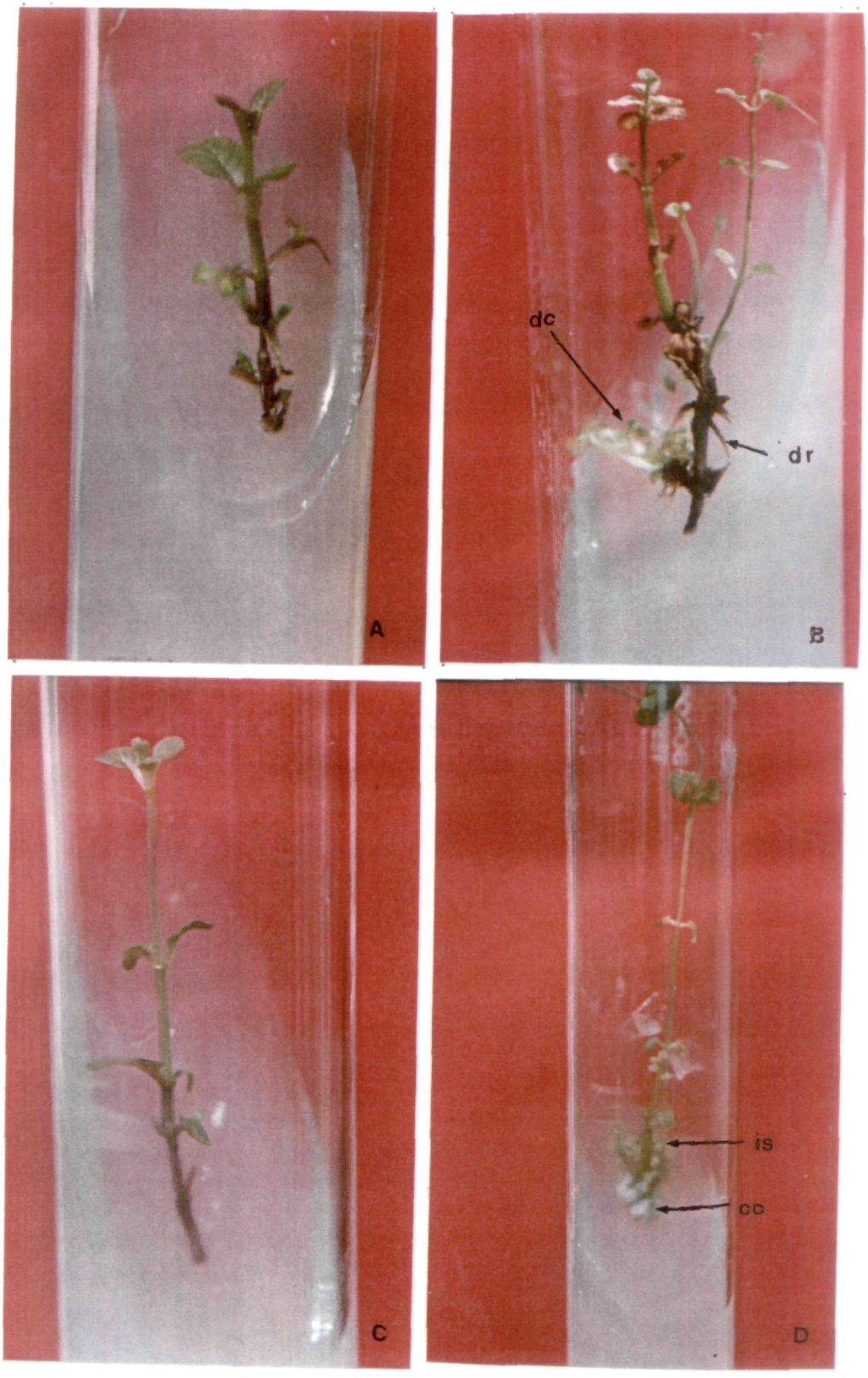
---

sb-shoot buds ; dc- direct caulogenesis ;

dr- direct rhizogenesis ; cc – compact callus ; is-indirect shoots.

Plate 6

Figs A--D



Explanation Of Plate –7

(Figs. A-D)

Fig A) 27 days old culture showing direct caulogenesis when shoot tip explant cultured on MS + Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$).

Fig B) 40 days old culture showing callogenesis, indirect rhizogenesis obtained from shoot tip explant cultured on MS + Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$).

Fig C) 18 days old culture showing direct caulogenesis from shoot tip explant cultured on MS + Kn ($2mgl^{-1}$) + IBA ($1mgl^{-1}$).

Fig D) 18 days old culture showing brown compact callus and indirect rhizogenesis obtained from shoot tip explant cultured on MS + IBA ($1.5mgl^{-1}$).

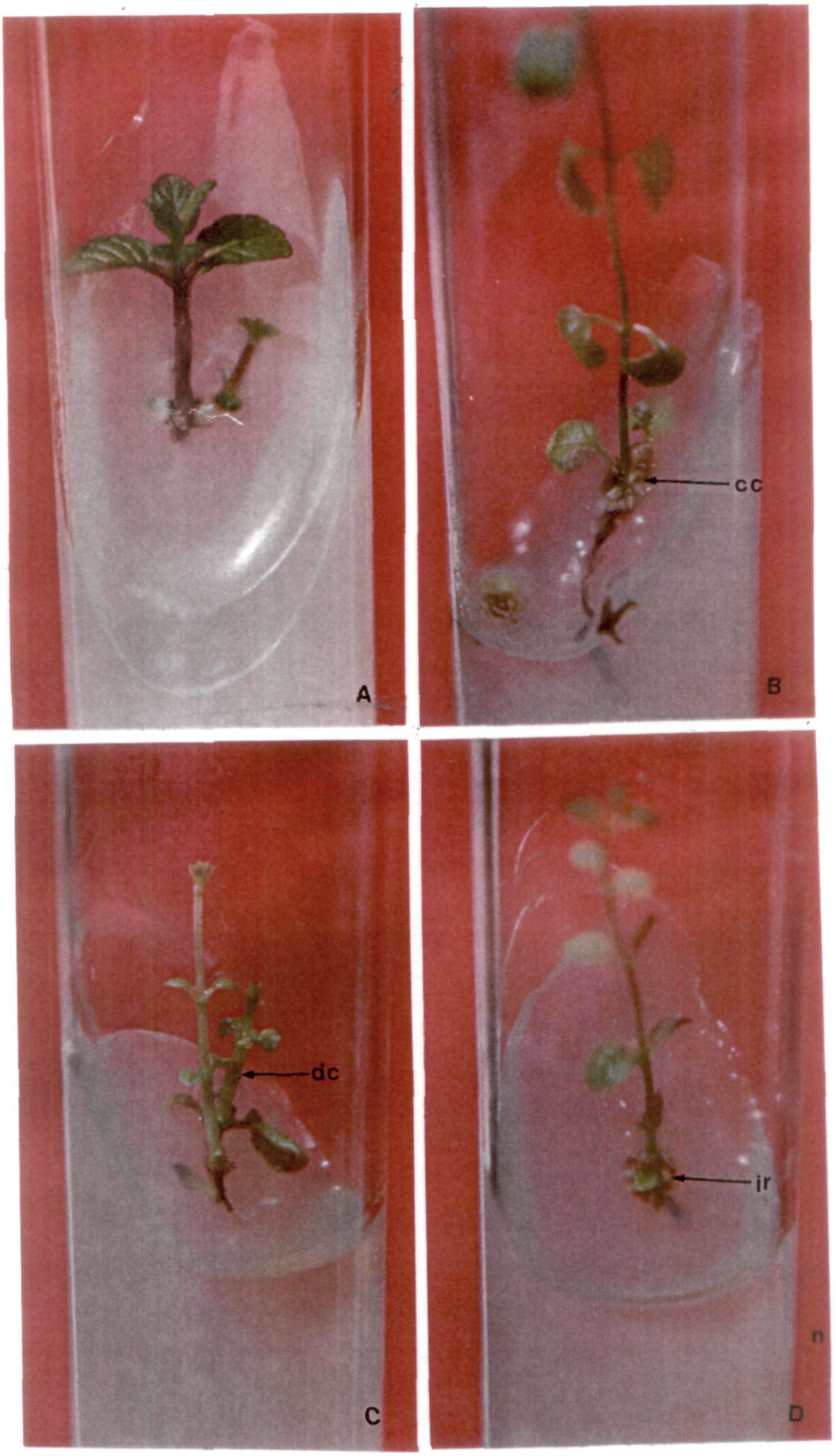
---

dc –direct caulogenesis ; cc compact callus ;

ir- indirect rhizogenesis.

Plate 7

Figs A–D



Explanation of Plate 8

(Figs. A-E)

Fig A) 44 days old culture showing emergence of direct shoots when shoot tip explant cultured on MS + Kn($2mgl^{-1}$) + NAA ($1mgl^{-1}$).

Fig B) 28 days old culture showing direct caulogenesis when shoot tip explant cultured on MS + Kn ($5mgl^{-1}$) + NAA ($.5mgl^{-1}$).

Fig C) 30 days old culture showing direct multiple shoot regeneration with small compact callus obtained from shoot tip explant cultured on MS + Kn ($5mgl^{-1}$) + NAA ($1mgl^{-1}$).

Fig D) 24 days old culture showing direct caulogenesis from shoot tip explant cultured on MS + Kn ($5mgl^{-1}$) + IAA ($.5mgl^{-1}$).

Fig E) 17 days old culture showing brown friable callus and indirect rhizogenesis from shoot tip explant cultured on MS + IBA ($1.5mgl^{-1}$).

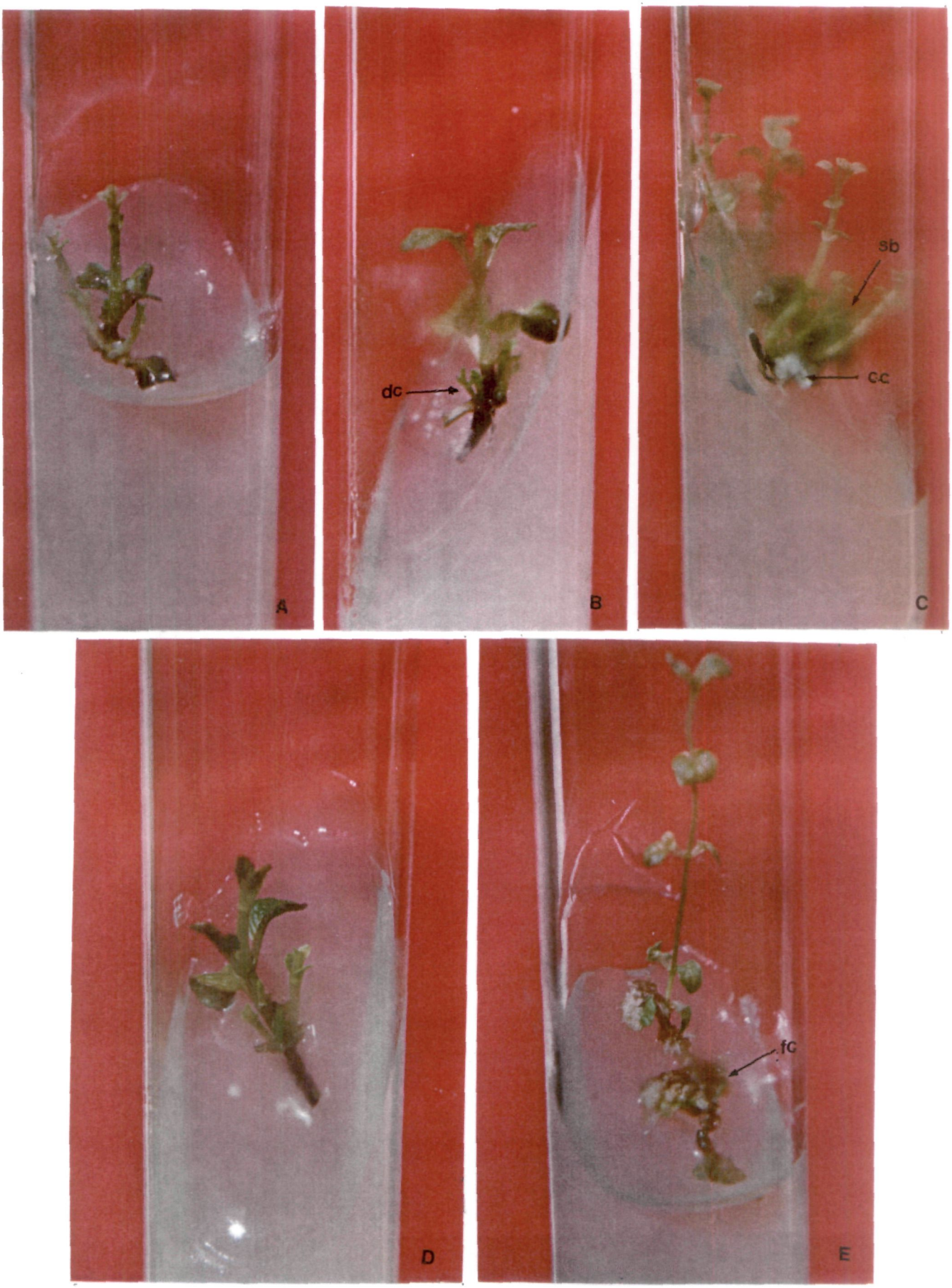
---

sb –shoot buds ; dc- direct caulogenesis ;

cc- compact callus ; fc- friable callus.

Plate 8

Figs A–E



Explanation of Plate 9

(Figs. A-E)

Fig. A) 33 days old culture showing direct caulogenesis from the cut end of shoot cultured on MS + BAP ($2mgl^{-1}$) + NAA ($.5mgl^{-1}$).

Fig. B) 45 days old culture showing direct caulogenesis along with well developed aerial root from shoot tip explant cultured on MS + BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$).

Fig. C) 43 days old culture showing direct caulogenesis, along with well developed aerial roots from shoot tip explant cultured on MS + BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$).

Fig. D) 23 days old culture exhibiting emergence of direct caulogenesis from shoot tip explant cultured on MS + BAP($2mgl^{-1}$) + IAA ($1mgl^{-1}$).

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dc- direct caulogenesis ; ar-aerial roots.

Plate 9

Figs A–D

Explanation of Plate 10

(Figs. A-E)

Fig. A) 28 days old culture showing direct caulogenesis from the cut end of the shoot tip explant cultured on MS + BAP ($5mgl^{-1}$) + NAA ($.2mgl^{-1}$).

Fig. B) 30 days old culture showing direct caulogenesis from the portion of the shoot tip explant cultured on MS + BAP ($2mgl^{-1}$) + Kn ($2mgl^{-1}$).

Fig.C) 18 days old subculture showing formation of multiple roots from compact brown callus cultured on MS + NAA ($2mgl^{-1}$).

Fig. D) 24 days old culture showing direct caulogenesis and rhizogenesis obtained from shoot tip explant cultured on MS + BAP ($2mgl^{-1}$) + NAA ($.5mgl^{-1}$).

Fig. E) *In vitro* raised plants acclimatized in plastic pot having mixture of soil and soilrite (3:1).

Fig. F) Healthy growing plantlets transferred to earthen pot

---

dc-direct caulogenesis ; cc- compact callus ;

ir- indirect rhizogenesis ; dr – direct rhizogenesis.

Plate 10

Figs A – F

Explantation of Plate 11

(Figs. A-F)

Fig. A) 25 days old culture showing induction of single direct root from nodal explant cultured on MS + CONTROL.

Fig.B) 24 days old culture showing direct caulogenesis from nodal explant cultured on MS + NAA ($2mgl^{-1}$).

Fig.C) 40 days old culture showing elongation of shootlets from nodal explant cultured on MS + IAA ($2mgl^{-1}$).

Fig.D) 28 days old culture showing direct caulogenesis and rhizogenesis from nodal explant cultured on MS + IAA ($4mgl^{-1}$).

Fig.E) 40 days old culture showing compact callus formation from nodal explant cultured on MS + Kn ($2mgl^{-1}$)

Fig.F) 42 days old culture showing compact callusing and formation of large number of shoots from nodal explant cultured on MS + BAP ($2mgl^{-1}$).

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r-root ; sh –shoot ; dc-direct caulogenesis ;
cc-compact callus ; dr-direct rhizogenesis.

Plate 11

Figs A – F

Explanation Of Plate 12

(Figs. A-H)

Fig.A) 32 days old culture showing formation of white compact callus from nodal explant cultured on MS + Kn (.5mgl^{-1}) + IBA(5mgl^{-1}).

Fig.B) 22 days old culture showing formation of multiple direct shoots from nodal explant cultured on MS + Kn (1mgl^{-1}) + NAA (2mgl^{-1})

Fig.C) 20 days old culture showing emergence of shoots from nodal explant cultured on MS + Kn (2mgl^{-1}) + 2, 4-D (1mgl^{-1}).

Fig.D) 17 days old culture showing direct caulogenesis from nodal explant cultured on MS + Kn (2mgl^{-1}) + NAA (1mgl^{-1})

Fig.E) 18 days old culture exhibiting direct multiple shoots from nodal explant cultured on MS + Kn (2mgl^{-1}) + IAA (1mgl^{-1}).

Fig. F) 20 days old culture showing initiation of direct multiple shoots from nodal explant cultured on MS + Kn (2mgl^{-1}) + IBA (1mgl^{-1}).

Fig.G) 32 days old culture showing formation of direct multiple shoots from nodal explant cultured on MS + Kn (2mgl^{-1}) + BAP (2mgl^{-1}) + IAA (.5mgl^{-1}).

Fig.H) Caulogenesis and rhizogenesis observed when nodal explant subcultured on MS + IBA (1.5mgl^{-1}), induction of single shoot observed on 17th day of transfer.

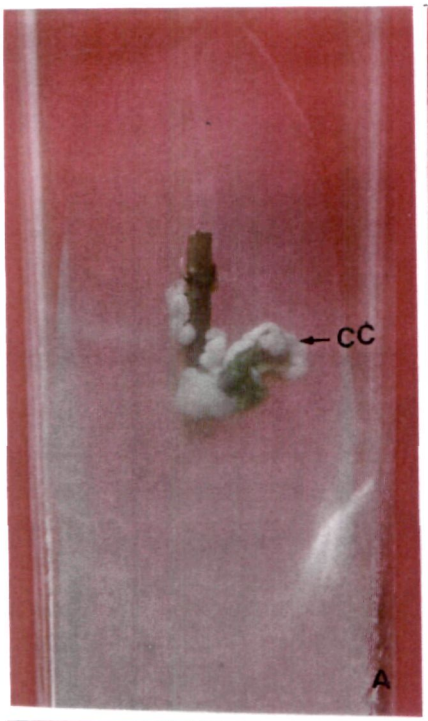
---

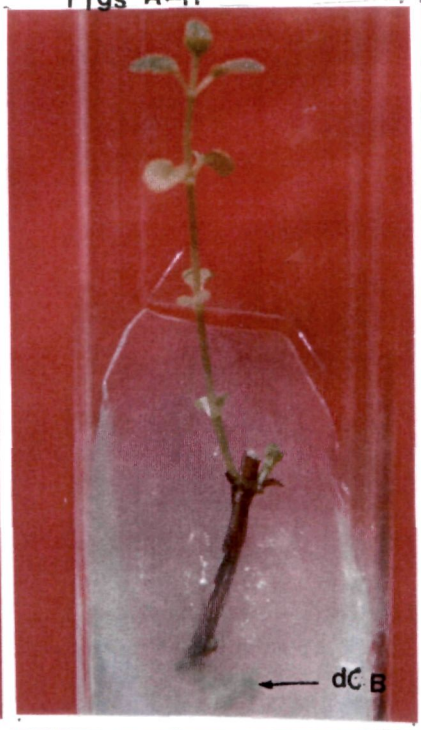
cc- compact callus ; dc-direct caulogenesis ;

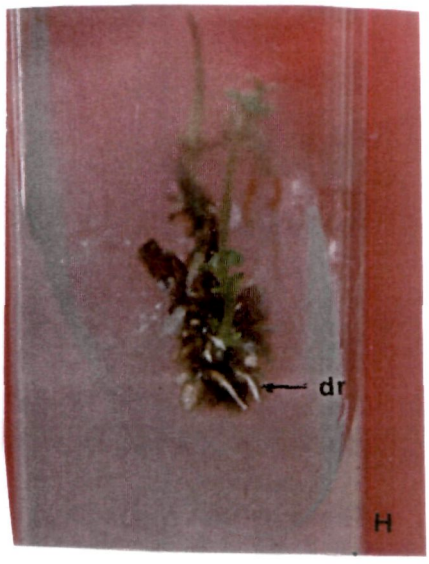
sh- shoot ; dr- direct roots.

Plate 12

Figs A–H







Explanation of Plate 13

(Figs. A-G)

Fig.A) Direct caulogenic response observed from nodal explant after 4 days of inoculation on MS + BAP ($2mgl^{-1}$) + 2, 4-D ($1mgl^{-1}$).

Fig.B) Nodal explant exhibited callogenesis and direct caulogenesis after 9 days of inoculation on MS + BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$).

Fig.C) Nodular callus exhibited from nodal explant after 9 days of inoculation on MS + BAP ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$)

Fig.D) Direct caulogenesis observed from nodal explant cultured on MS + BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$) after 23 days of inoculation.

Fig.E) Direct caulogenic response and rhizogenesis observed from nodal explant cultured on MS + BAP ($3.0\ mgl^{-1}$) + IAA ($1.5mgl^{-1}$) after 8 days of inoculation.

Fig.F) *In vitro* raised plants transferred to plastic pot containing mixture of soil and soilrite in the ratio of (3:1).

Fig.G) Successfully growing plantlets under field condition in earthen pots.

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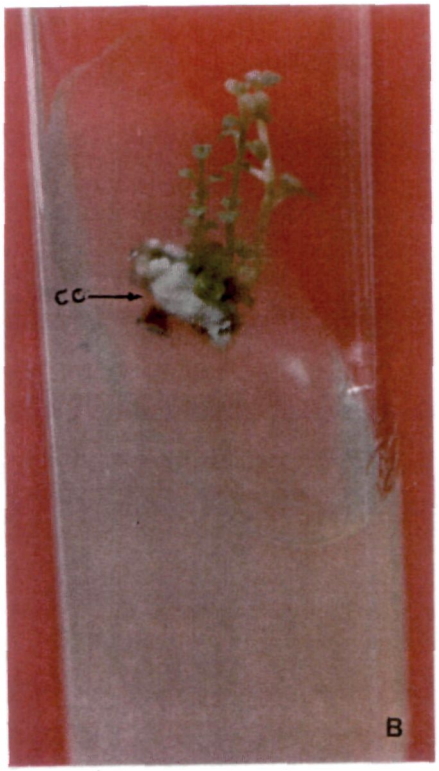
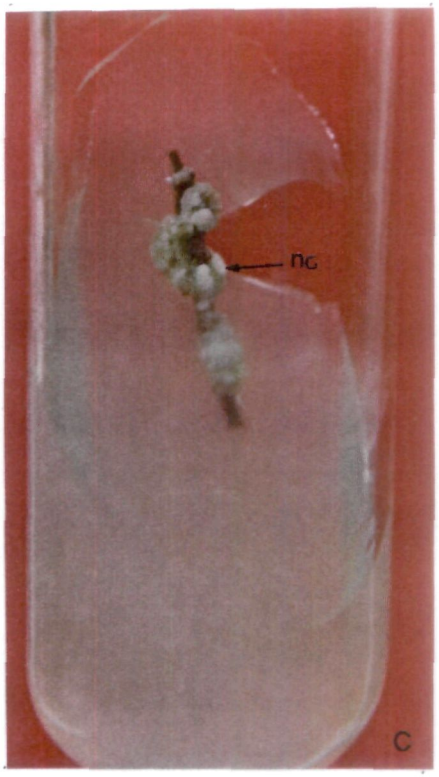
sh –shoot ; nc nodular callus ; cc-compact callus ;
dc –direct caulogenesis ; dr – direct rhizogenesis.

Plate 13

Figs. A – G





Explanation of Plate 14

(Figs. A-E)

Fig.A) 30 days old culture showing that leaf explant remained green in control medium.

Fig.B) 40 days old culture showing direct rhizogenesis from leaf explant cultured on MS + NAA ($2mgl^{-1}$).

Fig.C) 32 days old culture showing creamish green friable callus from leaf explant cultured on MS + NAA ($2mgl^{-1}$) + BAP ($3mgl^{-1}$).

Fig.D) 24 days old culture showing nodular dark yellow callus from leaf explant cultured on MS + Kn ($0.5mgl^{-1}$) + IBA ($5mgl^{-1}$)

Fig.E) 24 days old culture showing nodular creamish callus from leaf explant cultured on MS + Kn ($3mgl^{-1}$) + NAA ($1.5mgl^{-1}$).

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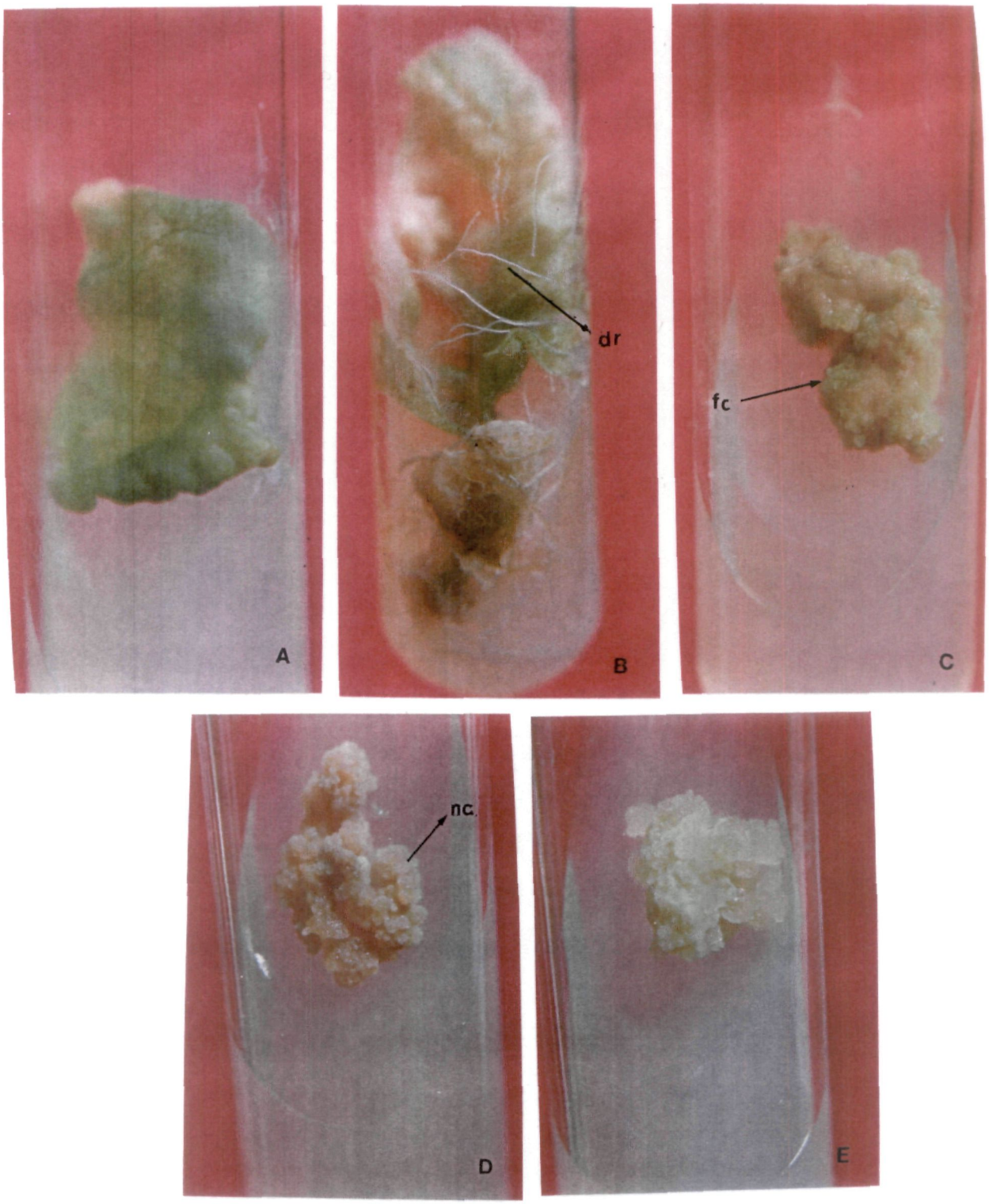
dr-direct rhizogenesis ; fc-friable callus ; nc -nodular callus.

Plate 14

Figs A–E



Explanation of Plate 15

(Figs. A.D)

Fig.A) 41days old culture showing yellow nodular callus along the margins of leaf lamina from leaf explant cultured on ½MS + Kn ($5mgl^{-1}$) + NAA ($0.5mgl^{-1}$).

Fig.B) 30 days old culture showing emergence of shoot bud initiation from brown leaf callus cultured on MS + BAP ($2mgl^{-1}$).

Fig.C) 41 days old culture showing whitish green compact callus obtained from leaf explant cultured on MS + BAP ($3mgl^{-1}$) + IAA ($2mgl^{-1}$).

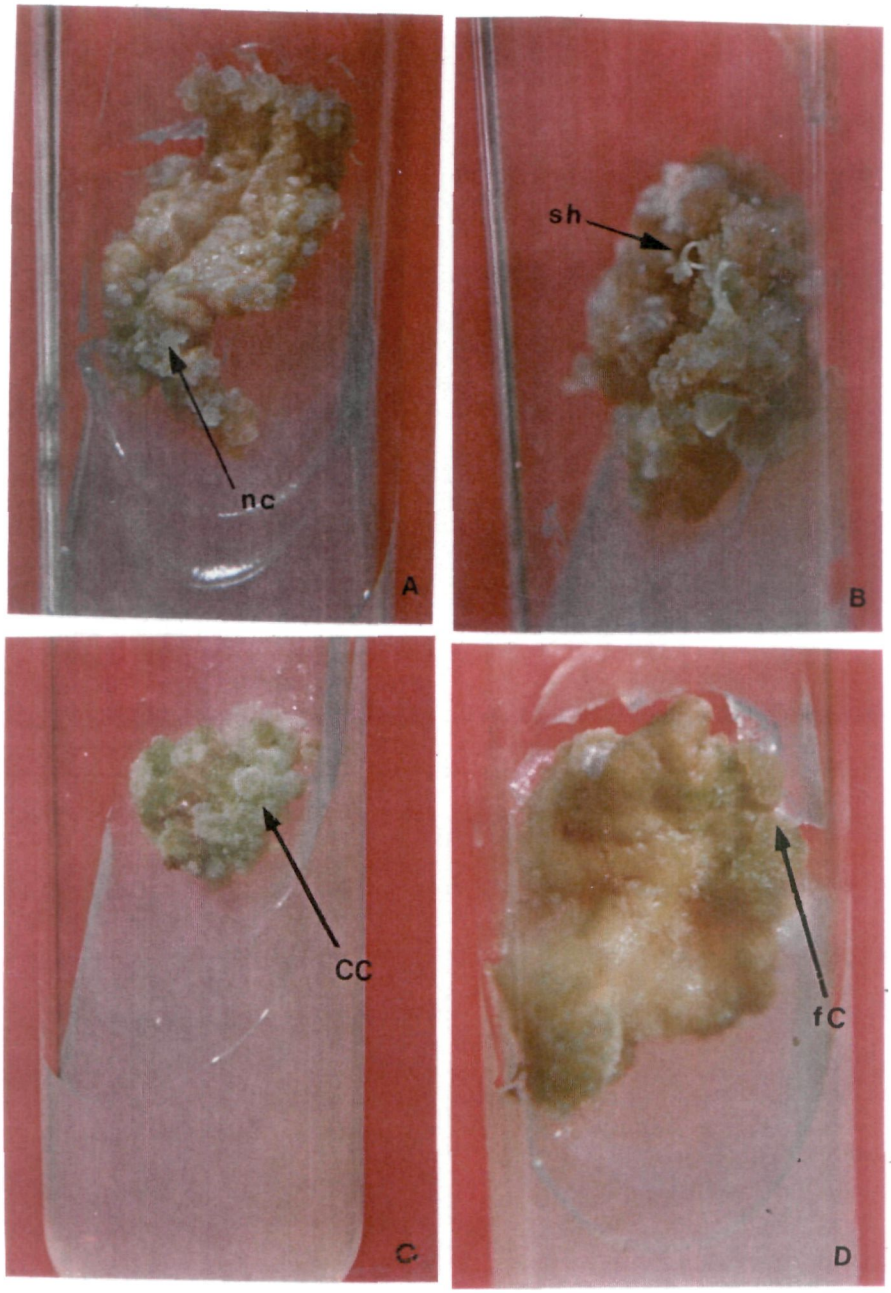
Fig.D) 32 days old culture showing yellowish green friable callus obtained from leaf explant cultured on MS + BAP ($2mgl^{-1}$) + Kn ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$).

---

nc-nodular callus ; cc-compact callus ;
fc-friable callus ; sh-shoot.

Plate 15

Figs A–D



Explanation of Plate 16

(Figs. A-E)

Fig.A) 42 days old culture showing direct caulogenesis alongwith well developed *in vitro* inflorescence from nodal explants cultured on MS + IBA ($2mgl^{-1}$).

Fig.B) 15 days old culture showing direct caulogenesis and *in vitro* inflorescence from nodal explants cultured on MS + IAA($1.5mgl^{-1}$).

Fig.C) 22 days old culture showing friable yellow callus along with *in vitro* inflorescence and direct rhizogenesis from nodal explants cultured on MS + IAA ($2mgl^{-1}$).

Fig.D) 30 days old culture showing formation of indirect rhizogenesis from brown friable callus obtained from nodal explants cultured on MS + IAA ($3mgl^{-}$).

Fig.E) 22 days old culture showing *in vitro* inflorescence, elongation of shoot & direct rhizogenesis from nodal explants cultured on MS+ IAA ($4mgl^{-1}$).

---

i – inflorescence ; fc-friable callus ;

ir-indirect rhizogenesis ; dr-direct roots.

Plate 16

Figs A – E

Explanation of Plate 17

(Figs. A-F)

Fig.A) 15 days old culture showing formation of greenish compact callus *in vitro* inflorescence and direct caulogenesis obtained from nodal explant cultured on MS+ BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$).

Fig.B) 17 days old culture showing formation of friable callus, *in vitro* inflorescence and direct caulogenesis from nodal explant cultured on MS + BAP ($2mgl^{-1}$) + IAA($1mgl^{-1}$).

Fig.C) 15 days old culture showing initiation of abrupt multiple shoot buds from nodal explant cultured on MS+Kn ($5mgl^{-1}$)+IAA($.5mgl^{-1}$).

Figs.Dand E) 35 days old culture showing complete plant regeneration when nodal explant cultured on MS+ IAA ($2mgl^{-1}$) + Kn ($1.5mgl^{-1}$).

Fig.F) Acclimatized plantlets.

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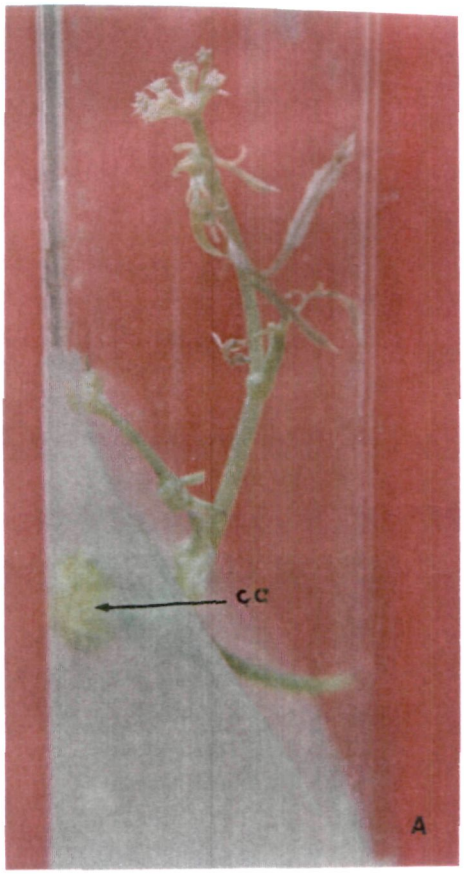
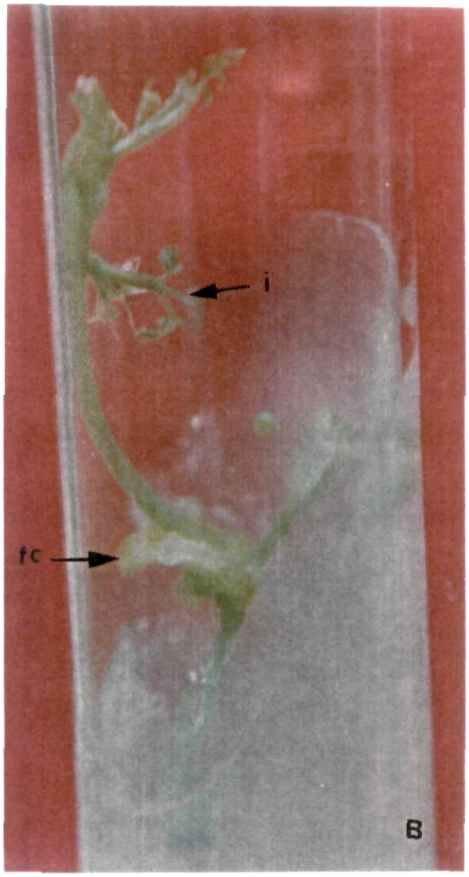
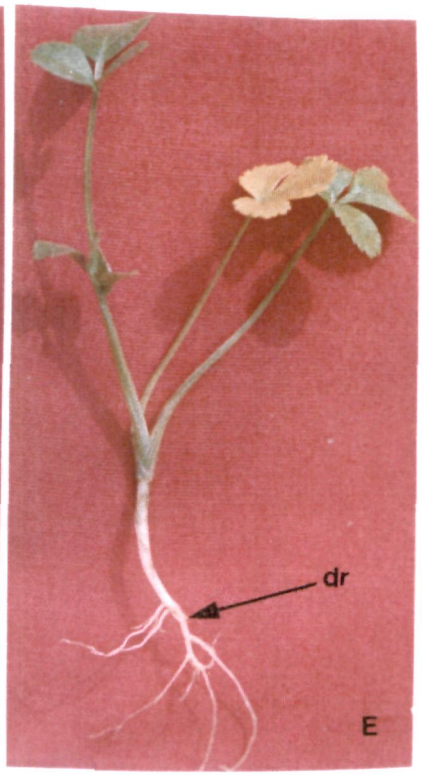
i-inflorescence ; fc-friable callus ; cc-compact callus ;
sb-shoot buds ; dr-direct rhizogenesis.

Plate 17

Figs A–F





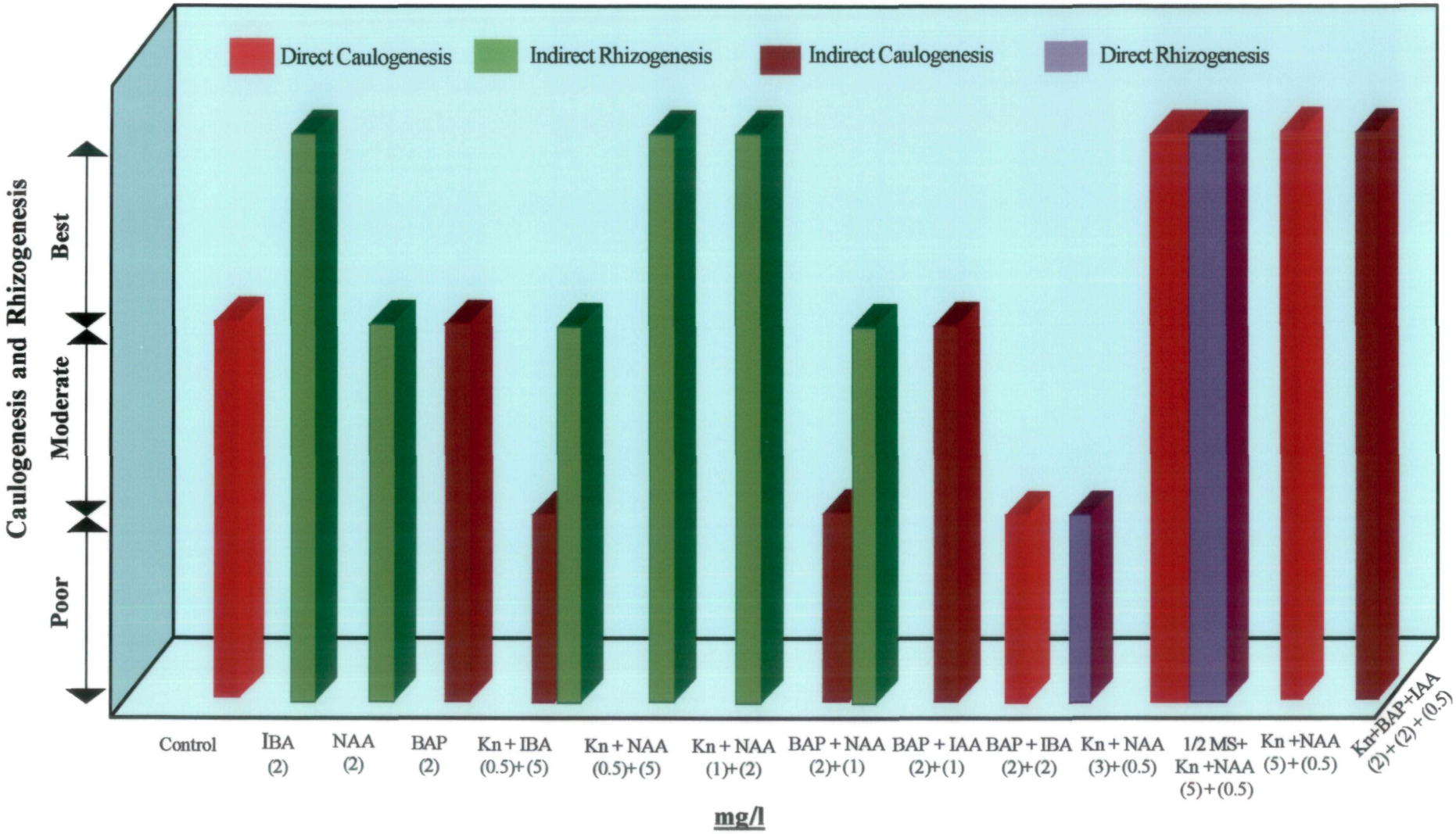


EXPLANATION OF HISTOGRAM 1

Histogram showing response of growth hormones
on Leaf explant of *Mentha arvensis* L.

HISTOGRAM 1

Response of growth hormones on Leaf explants of *Mentha arvensis* L.

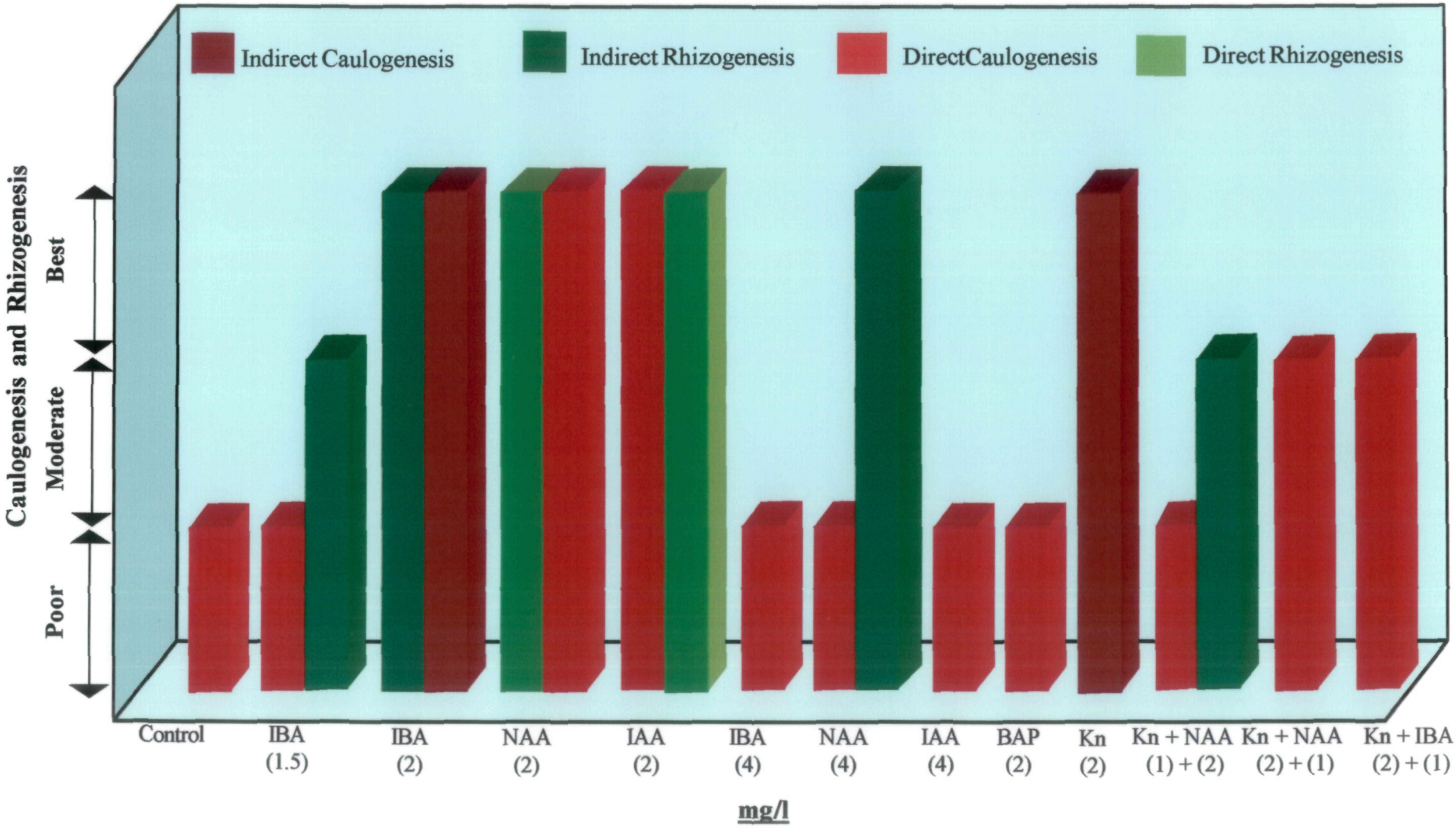


EXPLANATION OF HISTOGRAM 2

Histogram showing response of growth hormones on Shoot tip explant of *Mentha arvensis* L

HISTOGRAM-2

Response of growth hormones on Shoot tip explant of *Mentha arvensis* L.

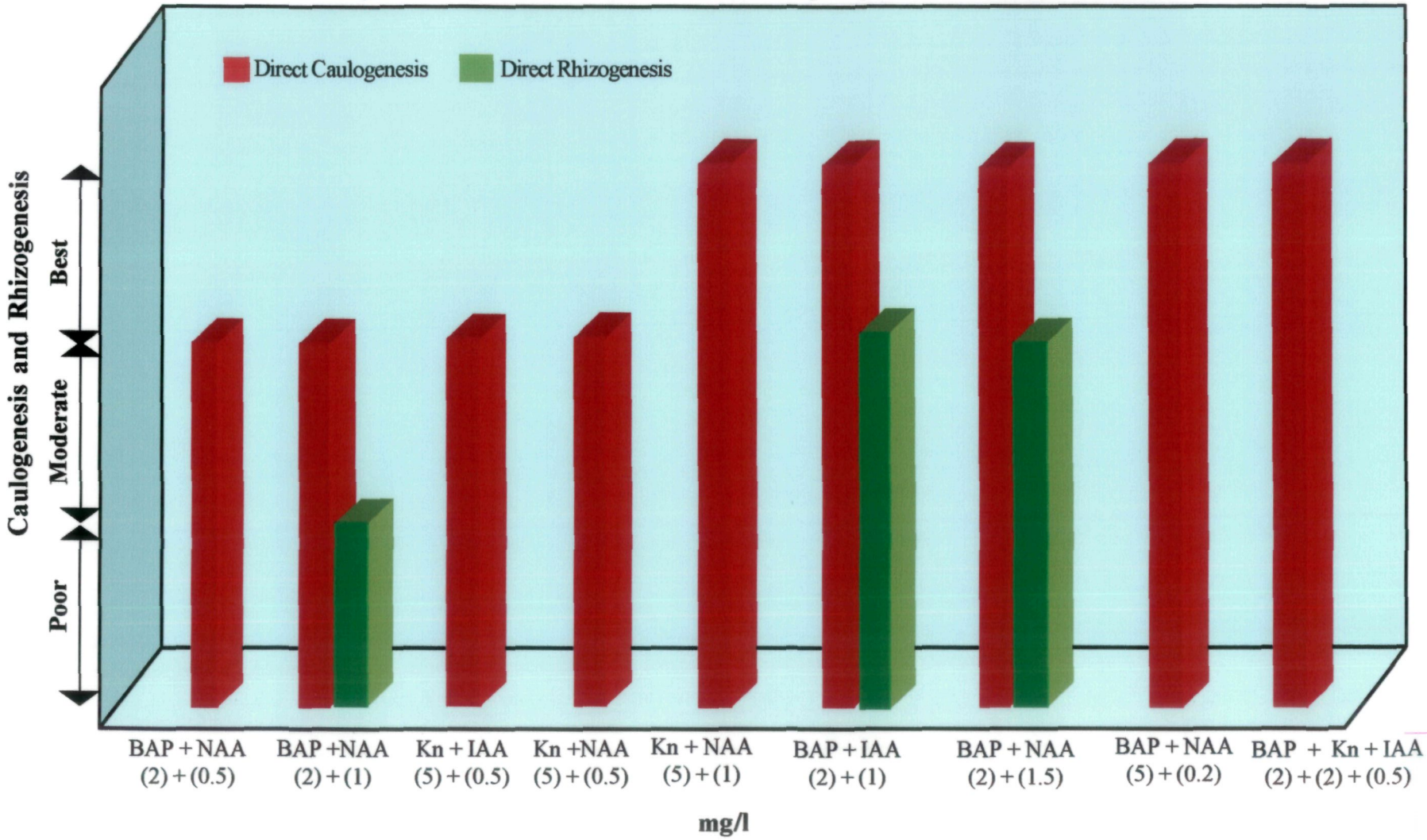


EXPLANATION OF HISTOGRAM 3

Histogram showing response of growth hormones on Shoot tip explant of *Mentha arvensis* L.

HISTOGRAM 3

Response of growth hormones on Shoot tip explants of *Mentha arvensis* L.

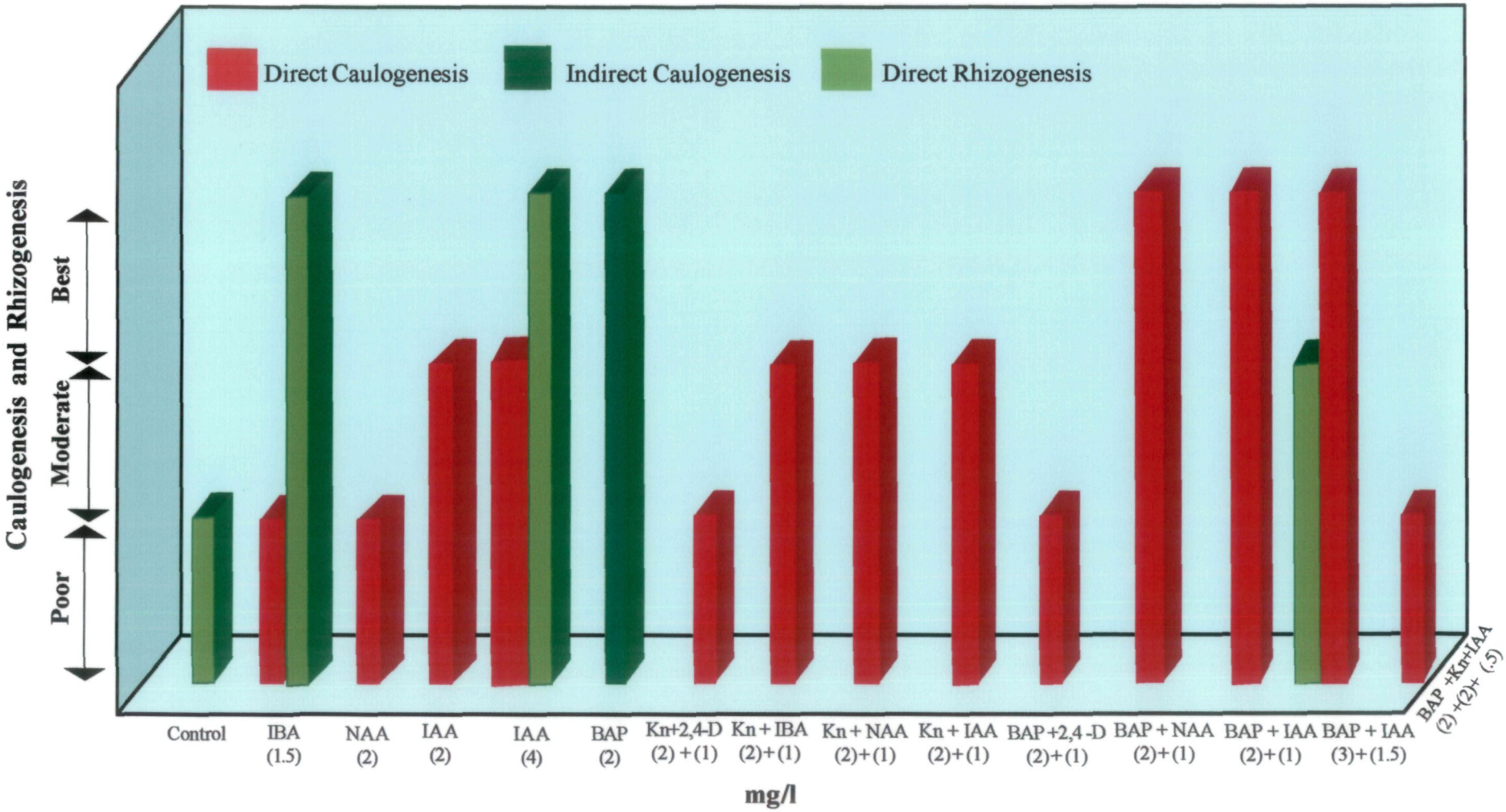


EXPLANATION OF HISTOGRAM 4

Histogram showing response of growth hormones on Nodal explant of *Mentha arvensis* L.

HISTOGRAM -4

Response of growth hormones on Nodal explants of *Mentha arvensis* L.

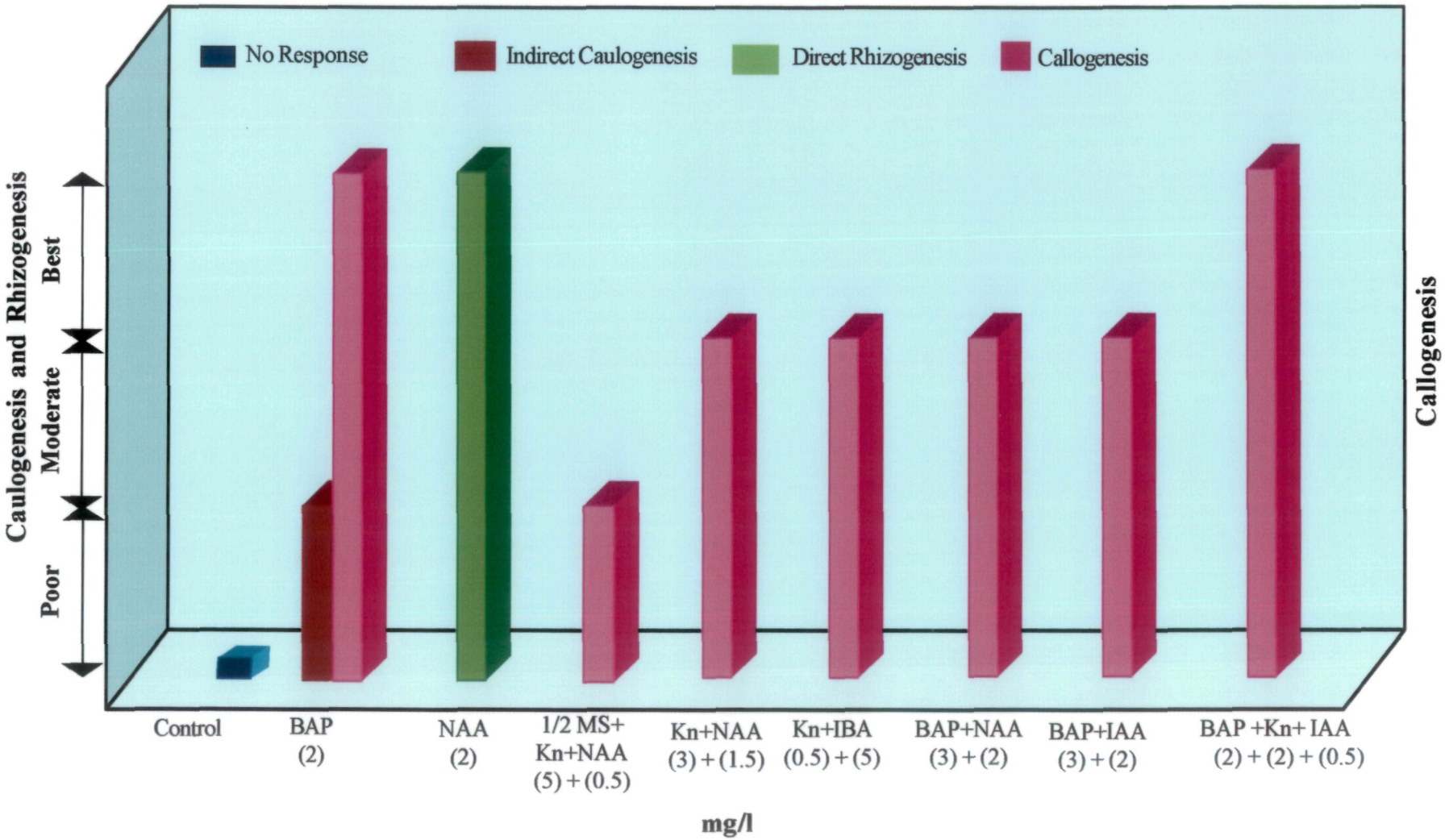


EXPLANATION OF HISTOGRAM 5

Histogram showing response of growth hormones on Leaf explant of *Ammi majus* L.

HISTOGRAM -5

Response of growth hormones on Leaf explants of *Ammi majus* L.

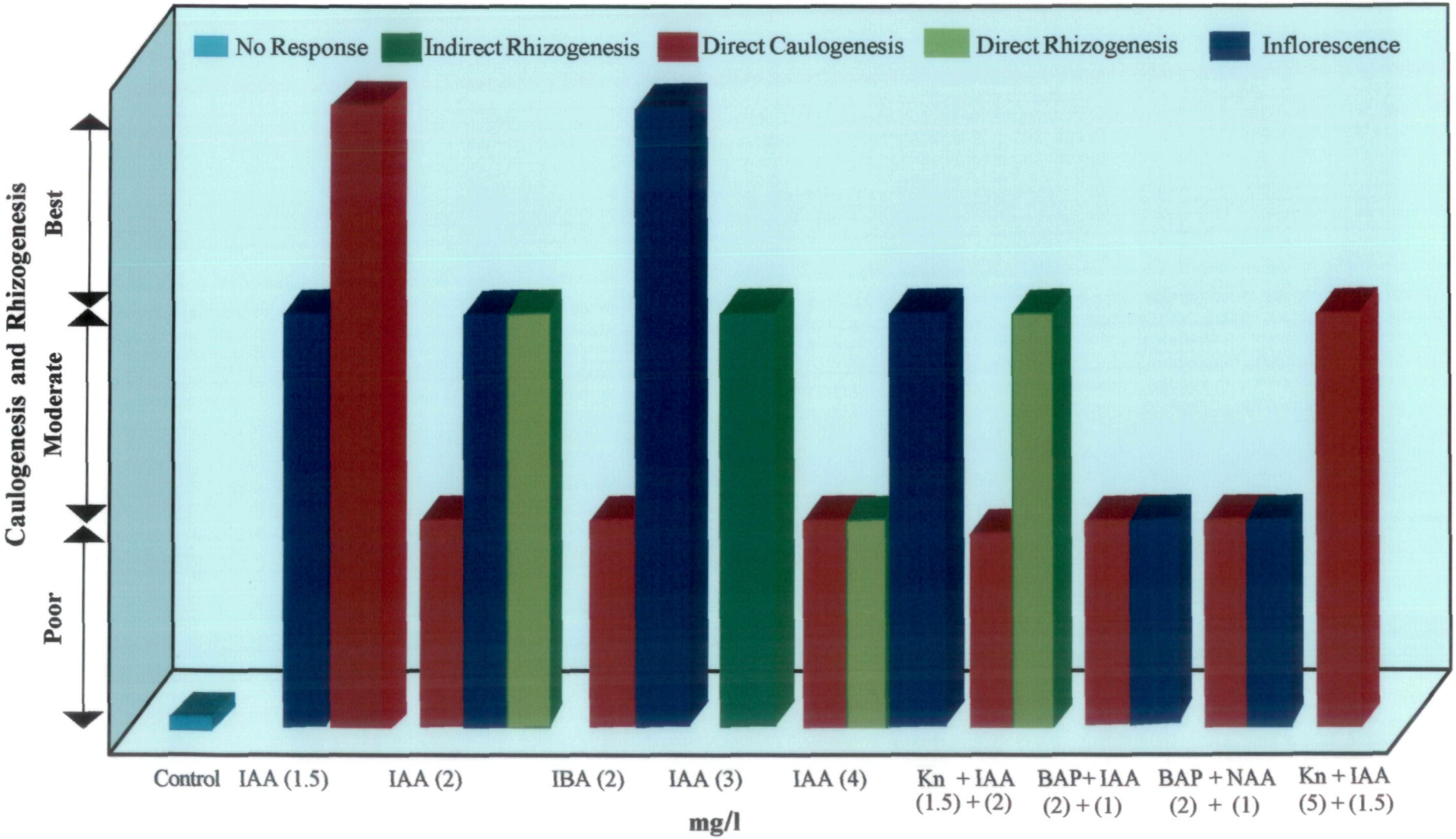


EXPLANATION OF HISTOGRAM 6

Histogram showing response of growth hormones
on Nodal explant of *Ammi majus* L

HISTOGRAM-6

Response of growth hormones on Nodal explants of *Ammi majus* L.



DISCUSSION

CHAPTER-5

DISCUSSION

The present work has been an endeavour to achieve rapid micropropagation of two economic medicinal crop plants. The results obtained have been discussed in the light of existing literature on the subject.

A successful procedure for direct and indirect regeneration and multiplication of *Mentha arvensis* L. from various explants was formulated.

Tissue culture provides an easy and inexpensive way for international exchange of disease free material (Murashige, 1977; Withers, 1980). However variation generated by the use of tissue culture regeneration techniques has resulted in improvement of diverse commercial crops (Reisch, 1983).

An alternative for improving mint may involve the use of tissue culture regeneration and the production of somaclonal variants (Larkin and Scowcroft, 1981). Todate orange mint and peppermint embryos (Van Eck and Kitto, 1990), Japanese mint have been proliferated *in vitro* rooted and acclimatised *in vitro*. The genotype dependent regeneration may be due to difference in genetic control of organogenesis by the different mint genotypes (Barocelli, 1974).

Among the three explant studied, leaf explant followed by shoot tip explant proved best for callus induction as reported earlier in *Mentha arvensis* (Khanuja *et al.*, 1998), (Bhaumik and Datta; 1988) and in *Mentha spicata* (Abou Mandour and Binder; 1998) (Li Xia *et al.*, 1999).

The doctrine of Skoog and Miller (1957) stated that both auxin and cytokinin are necessary for callus induction and regeneration which also holds good for *Mentha arvensis* as organogenic callus from shoot tip and nodal explants observed on NAA or IAA singly or in combination with

BAP/Kn. Several other workers also confirm it Khanuja *et al.*. (1998) and Xue Qi Han (1998) in *Mentha arvensis*. Similarly, Eck JM Van *et al.*. (1990) in *Mentha piperita* and Li Xia *et al.*. (1999) in *Mentha spicata*.

The present finding has clearly demonstrated that for indirect shoot regeneration. Leaf was the most responsive explant on BAP ($2mgl^{-1}$) alone or in combination with IAA ($1mgl^{-1}$) and or Kn ($2mg^{-1}$) which is in consonance with the earlier observations on BAP (44.4 (M) + cw (250ml/ litre) in *Mentha piperita* (Eck JM Van. et.al; 1992) Mints (Berry *et al.*; 1997) and in *Mentha pipertia*, spearmint (Faure *et al.*; 1998)

Increased morphogenic capacity of explant from the basal portion of leaves has been due to higher level of both IAA and absicissic acid (Rajasekaran *et al.*; 1987). In the same way only petiolar region of leaf was regenerated on IAA ($0.5mgl^{-1}$) but in combination with BAP ($2mgl^{-1}$) + Kn($2mgl^{-1}$). Leaf, shoot tip and nodal explants exhibited effective indirect caulogenesis on BAP ($2mgl^{-1}$) or Kn ($2mgl^{-1}$) alone or in combination with IAA ($1mgl^{-1}$)/IBA ($2mgl^{-1}$) it is in agreement with the findings obtained in *Mentha piperita* on BAP ($0.5mgl^{-1}$)+ NAA ($0.5mgl^{-1}$) by Eck JM Van *et al.* (1990), *Mentha spicata* on MS+NAA ($0.01mgl^{-1}$) Li Xia *et al.*. (1999) and *Mentha arvensis* piperascens on MS+NAA ($1mgl^{-1}$) + Kn ($1mgl^{-1}$) by Ono (1982).

It has been clearly demonstrated that for direct caulogenesis shoot tip explant and nodal explant was found to be most responsive which is in conformity with the results obtained in *Mentha piperita* (Rech and Pires 1986), Geslot *et al.* (1989), Pierik (1987).

The morphogenetic response of different explants with varied concentration of BAP was studied and it was observed that the number of shoots regenerated directly as well as indirectly, increases with an increase in concentration. This was confirmed by Rech and Pires (1986) from axillary buds of six *Mentha* sps. on BAP ($1,2mgl^{-1}$) + Kn ($1mgl^{-1}$), Rodov

and Davidova (1987) observed propagation of mint on LS + IBA ($0.1mgl^{-1}$) + GA ($0.1gml^{-1}$) + BAP ($0.5mgl^{-1}$) at low concentration.

Rhizogenesis is essential step for acclimatization of the regenerated plantlets into the field condition. In some plants rooting occurs on the same regeneration medium otherwise different media were used for root multiplication Mariska *et al.* (1987), Ravishankar and Venkataraman; 1988, Faure *et al.*; 1998, Ruseva; 1999. However, in some cases, shoots have to be separated and transferred to hormone free or half strength of MS medium as in present investigation.

Significant rhizogenesis was observed on half strength MS medium used. Same was taken into consideration by George and Ravishankar; 1996 in *Mentha* SPP. This may be due to presence of endogenous auxins which were sufficient to initiate rooting. The best rhizogenesis was induced with IBA, IAA alone at lower concentration whereas, in combinations higher auxin to lower cytokinin was required. Similar, results were obtained in Mint by Mariska *et al.* (1987) from stem explants on NAA ($0.5mgl^{-1}$). Rodov and Davidova; 1987, Ravishankar and Venkataraman; 1988, Eck JM Van *et al.* 1990, Faure; 1998 obtained results in consonance with the present findings on *Mentha piperita* but at lower concentration of auxins.

The findings of present workdone on *Ammi majus* L. demonstrates the possibility for propagation through the culture of leaf explants and nodal explants.

Response of shoot induction in *Ammi majus* was genotypic as well as tissue dependent, being good with nodal segment explant similar results were reported in *Foeniculum vulgare* Mill. by Theiler and Kagi (1992). In the present investigation among the two explants used, nodal explants proved best for direct shoot regeneration. The regeneration of shoot via callus form leaf explant was observed on BAP ($2mgl^{-1}$) in the present observation. Similar, results were obtained by Gosal *et al.* (1991) and Song

in *et al.* (1991) in stem segments of *Apium graveolens* and *Foeniculum vulgare* Gaertner. Madhumati *et al.* (1995) and Purohit *et al.* (1995) found similar results from cotyledonary leaves of *Ammi majus*.

Jay *et al.* (1994), Song *et al.* (1991) documented that the somatic embryogenesis was greatest in hormone free medium. However, in the present investigation leaf and nodal explant cultured on MS medium without hormones (control) fail to show any response.

Several studies made by several workers viz; Madhumati *et al.*; (1995). Purohit *et al.*; (1995) obtained callus from cotyledonary leaves in *Ammi majus* cultured on MS+IAA ($2mgl^{-1}$) + Kn ($5mgl^{-1}$) +CH ($1000mgl^{-1}$). Similar, results were reported in the present investigation on BAP ($2mgl^{-1}$) /Kn ($3,5mgl^{-1}$) in combination with IAA (1,2,3 mgl^{-1}), NAA (1,1.5, $0.5mgl^{-1}$) or IBA ($5mgl^{-1}$) and also different explants responded differently for callus induction.

Leaf explants produced nodular callus on BAP ($2mgl^{-1}$) alone or Kn (0.5,3,5 mgl^{-1}) in combination with IBA ($5mgl^{-1}$) or NAA (1.5, $0.5mgl^{-1}$) and friable callus on BAP ($3mgl^{-1}$) + NAA ($2mgl^{-1}$) or BAP ($2mgl^{-1}$) + Kn ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$). Similar, results were reported by Song *et al.* (1991) from leaf explant of *Foeniculum vulgare* Gaertner on 2,4-D ($0.01mgl^{-1}$) + BA (0.01-1.0 mgl^{-1}) While Madumati *et al.* (1995) and Purohit *et al.* (1995) obtained same results from cotyledonary leaves of *Ammi majus* on MS+IAA (2 mgl^{-1}) + Kn (5 mgl^{-1}) + CH (1000 mgl^{-1}).

In the present studies nodal explants that were slight tender and having greenish axillary buds responded efficiently for bud sprouting compared to hard nodal explants. Similar, findings were also documented in *Coriandrum sativum* (Kataeva *et al.*; 1993). Further Kataeva *et al.* reported stimulatory effect for micropropagation in *Coriandrum sativum* on MS+Kn or MS+Kn+IAA while in the present investigation IAA ($1.5mgl^{-1}$) proved best for sprouting from nodal explants.

Generally, a cytokinin or a combination of a cytokinin and auxin is required for *in vitro* shoot proliferation (Thorpe and Patel, 1984). Best results of shoot proliferation from axillary buds of *Ammi majus* were obtained on MS supplemented with IAA ($1.5mgl^{-1}$) followed by Kn ($5mgl^{-1}$) + IAA ($0.5mgl^{-1}$) was adequate to induce bud break and support subsequent shoot growth in the cultures of nodal explants these results are in confirmity with the results obtained by Kataeva *et al.* (1993) in *Coriandrum sativum* on MS+Kn or MS+Kn+IAA.

Song *et al.*. (1991) reported that low concentration of 2,4-D ($0.01mgl^{-1}$)+BA ($0.01mgl^{-1}$) stimulate callus induction and somatic embryogenesis from leaf and stem explants of *Foeniculum vulgare* Geartner. While Jager *et al.*. (1993) obtained callus culture of *Thapsia garganica* at low conc. of 2,4-D ($1mgl^{-1}$). Similarly, Schiava *et al.*. (1992) reported that low conc. of 2,4-D (0.1,30 mgl^{-1}) + BA ($0.25mgl^{-1}$) induced embryogenesis in *Daucus carota*. While 2,4-D in the present investigation fails to show any response from nodal as well as from leaf explant of *Ammi majus*.

In the present investigation nodal explants cultured in various combinations induce callus formation. Later on after subculturing on different medium i.e. BAP ($2mgl^{-1}$) shoot bud intiation was reported which is in consonance with the findings reported by Madhumati et *al.* (1995), Purohit *et al.* (1995) in the same plant *Ammi majus* using same combinations i.e. MS + αIAA ($2mgl^{-1}$) + Kn ($5mgl^{-1}$) + CH (1000 mgl^{-1}) adenine.

In vitro flowering was observed from nodal explant of *Ammi majus* on MS medium supplemented with IBA ($2mgl^{-1}$), IAA (1.5, 2.0,4.0 mgl^{-1}), BAP ($2mgl^{-1}$) +NAA ($1mgl^{-1}$), BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$). Similar, results were obtained by Purohit *et al.*. (1995) in same plant on MS fortified with IBA + glutamine.

NAA ($2mgl^{-1}$), IAA (2,3,4,mgl^{-1}, IAA ($2mgl^{-1}$)+Kn ($1.5mgl^{-1}$) are the most frequently used to effect rhizogenesis in the present investigation these findings are in consonance with the results obtained in *Daucus carota* on Miller's modified medium + NAA (0, 0.5, 1.0,1.5 or 2.0 p.p.m) + MBC [Methyl benzimidazolyl carbomate] (carbondazin) (0,2,4,8 or 10 p.p.m) Laxmi *et al.*. (1991). While Madhumati *et al.*. and Purohit *et al.*. (1995) documented that the presence of MS+IBA+ glutamine was necessary for rhizogenesis in *Ammi majus*.

In the present study, proliferation and maintenance of callus was found responding in different growth hormiones in different ways and the medium studied here is found more suitable for the purpose.

The method of plant regeneration from calli stem segments and leaf insure a constant supply of plant material which are of immense scientific utility and our results may provide an efficient dependable protocol to *in vitro* methods for rapid propagation of elite genotypes of *Mentha arvensis* L. and *Ammi majus* L.

SUMMARY

CHAPTER-6

SUMMARY

The morphogenetic potential of two economically important plants i.e. *Mentha* arvensis L. and *Ammi majus* L. have been investigated in the present study. The results are summarized as follows;

6.1 *Mentha arvensis* L.

The leaf, shoot tip and nodal explants were cultured on MS basal medium to induce callus and shoot bud differentaition either directly or indirectly.

6.1.1 Callogenesis:

Leaf explants cultured on MS basal medium enriched with IBA ($2mgl^{-1}$), NAA ($2mgl^{-1}$), BAP ($2mgl^{-1}$)+NAA ($1mgl^{-1}$), Kn ($0.5mgl^{-1}$) + IBA ($5mgl^{-1}$) exhibited brownish, compact callus whereas, white compact callus was obtained when the medium was fortified with various phytohormones viz; BAP ($2mgl^{-1}$), BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$), BAP ($2mgl^{-1}$) + 2,4-D ($1mgl^{-1}$), Kn ($0.5mgl^{-1}$) + NAA ($5mgl^{-1}$), Kn ($2mgl^{-1}$)+ 2,4-D ($1mgl^{-1}$), Kn ($3mgl^{-1}$) + NAA ($0.5mgl^{-1}$), 1/2 MS+Kn ($5mgl^{-1}$) + NAA ($0.5mgl^{-1}$), Kn ($2mgl^{-1}$)+BAP ($2mgl^{-1}$)+ IAA ($0.5mgl^{-1}$) the same explant when cultured on control produced brownish white friable callus and brownish friable callus was produced on Kn ($1mgl^{-1}$) + NAA ($2mgl^{-1}$), Kn ($0.5mgl^{-1}$) + IBA ($5mgl^{-1}$).

The shoot tip explant when cultured on MS medium enriched with IBA($1.5,2mgl^{-1}$), NAA($2,4mgl^{-1}$), Kn($1mgl^{-1}$) + NAA($2mgl^{-1}$) showed brown compact callus whereas, white compact callus was obtained when MS medium fortified with Kn ($2mgl^{-1}$), Kn($5mgl^{-1}$)+ NAA($1mgl^{-1}$). The same explant when cultured on IBA($1.5mgl^{-1}$) exhibited brown friable callus.

The nodal explants when cultured on Kn ($2mgl^{-1}$) + BAP ($2mgl^{-1}$), Kn ($0.5mgl^{-1}$) + IBA ($5mgl^{-1}$), BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$) exhibited white

compact callus. White nodular callus was produced form nodal explants cultured on BAP ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$).

6.1.2 Caulogenesis:

Indirect multiple shoot regeneration was obtained from leaf explants cultured on Kn ($2mgl^{-1}$) + BAP ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$) while moderate indirect caulogenesis from leaf explant was observed from BAP ($2mgl^{-1}$), BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$) and poor response was noticed on BAP ($2mgl^{-1}$)+ NAA ($1mgl^{-1}$), Kn ($0.5mgl^{-1}$) +IBA ($5mgl^{-1}$).

Similarly, shoot tip explants showed best response for indirect multiple shoot regeneration when cultured on IBA ($2mgl^{-1}$), Kn ($2mgl^{-1}$) alone.

Whereas, the nodal explants exhibited best response for indirect caulogenesis with BAP ($2mgl^{-1}$) alone.

The leaf explant cultured on control medium showed moderate response for direct caulogenesis. The same explant cultured on BAP ($2mgl^{-1}$) + IBA ($2mgl^{-1}$) exhibited poor direct caulogenesis. While on medium fortified with Kn ($5mgl^{-1}$)+ NAA ($0.5mgl^{-1}$) and 1/2MS+Kn ($5mgl^{-1}$)+NAA ($0.5mgl^{-1}$) leaf explants showed best caulogenic response directly.

Shoot tip explants proved better than nodal and leaf explants for direct caulogenesis. The maximum number of shoots were produced form shoot tip explants when grown on NAA ($2mgl^{-1}$), IAA ($2mgl^{-1}$), Kn ($5mgl^{-1}$) + NAA ($1mgl^{-1}$), BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$), BAP ($2mgl^{-1}$) + Kn ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$), BAP ($2,5mgl^{-1}$)+NAA (1.5, $0.2mgl^{-1}$) respectively. Average number of shoots were obtained from shoot tip explant grown on Kn ($2mgl^{-1}$) +IBA ($1mgl^{-1}$), Kn ($2mgl^{-1}$)+NAA ($1mgl^{-1}$), Kn($5mgl^{-1}$) + NAA ($0.5mgl^{-1}$), Kn ($5mgl^{-1}$) +IAA ($0.5mgl^{-1}$), BAP ($2mgl^{-1}$)+ IAA / NAA ($1mgl^{-1}$) and lesser number of direct shoots were observed on control medium, IBA ($1.5,4mgl^{-1}$), NAA ($4mgl^{-1}$), IAA ($4mgl^{-1}$), BAP ($2mgl^{-1}$).

Nodal explant cultured on MS medium fortified with BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$), BAP ($2,3mgl^{-1}$) + IAA (1, $1.5mgl^{-1}$) / NAA ($1mgl^{-1}$), showed best response for direct caulogenesis while moderate response was observed with treatment IAA (2,4 mgl^{-1}), Kn ($1,2mgl^{-1}$) + NAA ($2,1mgl^{-1}$), Kn ($2mgl^{-1}$) + IAA / IBA ($1mgl^{-1}$) respectively and lesser number of direct shoots were obtained from nodal explant cultured on IBA ($1.5mgl^{-1}$), NAA ($2mgl^{-1}$), BAP ($2mgl^{-1}$) + 2,4-D ($1mgl^{-1}$), Kn ($2mgl^{-1}$) + BAP ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$).

6.1.3 Rhizogenesis

Indirect profuse rhizogenesis was obtained through leaf explants cultured on IBA ($2mgl^{-1}$), Kn ($0.5,1mgl^{-1}$)+NAA ($5,2mgl^{-1}$) respectively and Kn(0.5, mgl^{-1}) + IBA ($5mgl^{-1}$) followed by NAA ($2mgl^{-1}$), BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$), Kn ($0.5mgl^{-1}$)+IBA ($5mgl^{-1}$) while best direct rhizogenesis was obtained when cultured on 1/2 MS medium fortified with Kn ($5mgl^{-1}$) + NAA ($0.5mgl^{-1}$) and poor direct rhizogenesis was noticed on MS + Kn ($3mgl^{-1}$) + NAA ($0.5mgl^{-1}$). Shoot tip explants exhibited maximum rhizogenic response directly on MS + NAA / IAA ($2mgl^{-1}$) followed by BAP ($2mgl^{-1}$) +IAA / NAA ($1,1.5\ mgl^{-1}$) poor direct rhizogenesis was noticed on MS+BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$). Nodal explants showed no sign of indirect rhizogenesis but best direct rhizogenesis was noticed on MS+IBA/IAA (1.5, $4mgl^{-1}$) while moderate direct rhizogenesis was observed on MS + BAP ($3mgl^{-1}$) + IAA ($1.5mgl^{-1}$) and poor direct rhizogenesis was noticed on control medium.

6.2 *Ammi majus* L.

Callus induction and organogenesis was observed from leaf and nodal explant cultured on MS medium supplemented with various growths hormones.

6.2.1 Callogenesis

Leaf explants showed creamish green friable callus on MS + NAA ($2mgl^{-1}$) + BAP ($3mgl^{-1}$) and yellow green friable callus on MS + BAP ($2mgl^{-1}$) + Kn ($2mgl^{-1}$) + IAA ($0.5mgl^{-1}$). While the nodal explant produced yellow friable callus on MS + IAA ($2mgl^{-1}$), brown friable callus on MS + IAA ($3mgl^{-1}$) and light green friable callus on MS + BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$). The same explant when cultured on MS + BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$) produced greenish compact calli. The leaf explants exhibited very good response for nodular callusing on MS + Kn($0.5mgl^{-1}$)+IBA ($5mgl^{-1}$) and MS+ Kn ($5mgl^{-1}$) + NAA ($0.5mgl^{-1}$) exhibited yellow nodular calli but on MS+Kn ($3mgl^{-1}$) +NAA ($1.5mgl^{-1}$) creamish nodular calli were obtained.

6.2.1 Caulogenesis:

Leaf explants showed poor response for indirect caulogenesis with medium having BAP ($2mgl^{-1}$) and no signs of direct caulogenesis were observed on leaf explant. Whereas, nodal explants showed no signs of indirect caulogenesis. The same explant when cultured on MS basal medium with IAA ($1.5mgl^{-1}$) exhibited best response for direct caulogenesis along with moderate inflorescence whereas, moderate direct caulogenesis was observed when cultured on MS basal medium supplemented with Kn ($5mgl^{-1}$)+IAA($0.5mgl^{-1}$), MS fortified with IBA ($2mgl^{-1}$), IAA ($2,4mgl^{-1}$), IAA($2mgl^{-1}$) + Kn ($1.5mgl^{-1}$), BAP ($2mgl^{-1}$)+NAA($1mgl^{-1}$) and BAP ($2mgl^{-1}$)+ IAA ($1mgl^{-1}$) exhibited poor response for direct caulogenesis along with inflorescence formation. Best inflorescence was noticed on MS+IBA ($2mgl^{-1}$). While moderate inflorescence was observed on IAA (1.5, 2,4-Dmgl^{-1}). Poor response was noticed in combination treatment viz; BAP ($2mgl^{-1}$) + NAA ($1mgl^{-1}$), BAP ($2mgl^{-1}$) + IAA ($1mgl^{-1}$).

6.2.3 Rhizogenesis:

Direct profuse rhizogenesis was obtained from leaf explant cultured on MS enriched with NAA ($2mgl^{-1}$) but indirect rhizogenesis could not be achieved through leaf explant while indirect moderate rhizogenesis was obtained from nodal explant cultured on MS+IAA ($3mgl^{-1}$). Nodal explants cultured on MS+IAA ($2mgl^{-1}$) or in combination i.e. MS fortified with IAA ($2mgl^{-1}$) + Kn ($1.5mgl^{-1}$) exhibited moderate direct rhizogenesis and poor direct rhizogenesis was observed on MS supplemented with IAA ($4mgl^{-1}$).

7.0 ACCLIMATIZATION OF PLANTLETS

Plantlets with well differentiated shoots and roots were transferred to a plastic pot containing sterile mixture of soil and soilrite 3:1 ratio covered with polythene bags in growth chamber 25±2°C for 15 days and then transferred to earthen pots containing pure garden soil and reared to maturity.

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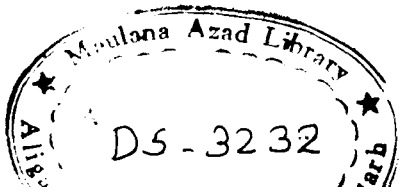

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